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GALVESTON

MILITARY MEDICAL SERVICE WITH SPECIAL EMPHASIS ON TROPICAL DISEASES

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It is not my intention to discuss technically any disease conditions, as I know such matters may be better approached in another manner. What I have to say should be regarded simply as an introduction to the consideration of certain phases of medical practice that we, as military men, must recognize as being of great importance, with the country engaged, as it is, in the most terrible war of all time.

The influence of the impact of war on the life of a nation is of such widespread effect, and introduced so violently, that the normal economy of the people is certain to experience serious disturbance. This disruption may be brought about by several factors: by the movement of men and materials to unaccustomed channels; by the development of new industries; by the greatly increased importance suddenly given practices perhaps little used in peacetime; and by the general diversion of talents from routine occupations and their application to those matters more intimately connected with the conduct of the war. Every person and all material things in the land will be caught in the net, because war leaves no home or institution untouched, no pleasure or vocation unaffected. Farm, factory, the ships of trade, the very vehicles provided for our pleasure, all became part of the material demanded by the exigencies of war. The artisan, the actor, the farmer, the clerk and the professional man, the housewife and the student are affected alike; and the lives of all are altered to a degree proportionate to the extent of the military effort required to insure the safety of the nation.

This thought comes home to us with peculiar force, because among the arts and sciences, none is more profoundly disturbed in time of war than that of medicine. No profession gives more to the cause of war and none derives greater good from the fact of war than ours. During the past several hundred

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years, ever since an enlightened public consciousness began to demand the establishment of medical service for the care of soldiers, the role of medicine has become of increasingly great importance to the maintenance of armies in the field. The importance of the contribution that medicine is able to make to the military effort rests not only on the fact that, by the application of proper remedial measures, the maintenance of armies in the field is supported by the restoration to duty of sick and wounded; nor on the strengthening of morale in the soldier through the knowledge that he will be adequately cared for if disabled; but it also depends very largely upon the application of recognized rules of preventive medicine.

The compelling influence of preventive medicine in military life cannot be overemphasized, because the primary function of the Medical Department in war is the conservation of manpower, a very different concept of medicine than that habitually entertained by the physician toward his patient. The military medical officer must, through the very nature of his occupation and the necessities of warfare, think of his patient not only as a suffering human being, entitled to such comfort as medical skill may make possible; he must also look upon him as an integral part of the war machine and as such, he is a useless member of the military establishment every moment that his physical or mental condition serves to remove him from the sphere of active military duty. In respect to these matters, the medical officer, like his colleague in civilian practice, is the final authority in all matters concerned with diagnosis and treatment, but in his military relationship to the commander, his status is that of an adviser.

So far as professional knowledge and humanitarian considerations may permit, medical thought in the service must conform to the military plans of the commander, and the medical officer must constantly bear in mind the thought that his first duty is to keep the soldier fit for active service; and next, to restore to duty at the earliest possible date those who have become the victims of injury or disease.

The formation of a vast Army and Navy, such as America is now building, cannot help but result in a profound change in the thought and practices of the medical profession of the

country. Enormous numbers of physicians are being drawn from their occupations in civil life for service with the military; neighborhoods have been in many instances seriously depleted of physicians. No medical school of standing has escaped the effect of this withdrawal of medical talents and every institution in the land has, as a contribution to the war effort, released many of its most competent instructors to the service. The medical profession cannot escape the implications and the effects of war; nor can it avoid consideration of the medical problems that spring out of war.

We are well aware that the enormous concentrations of young men brought about in camps throughout the country immediately raises problems of the first importance from the medical viewpoint. Knowing that the rapid gathering together in one spot of large groups of youngsters from widely separated sections of the country will almost certainly serve to introduce in the camps many sorts of infectious diseases from specific localities, the thoughtful minds in the profession immediately begin to think seriously of the possible dangers to our Army and to our civil population through the development of epidemic diseases.

The bitter memory of the last war, when so many, many thousands of lives were lost during the influenza epidemic, is still fresh in the minds of those who lived through that dreadful period. While, at the outbreak of the present war, there was no reason to believe a similar experience awaisted us, thought had to be given to the strong probability that there would be outbreaks of many other disabling diseases. Measles, mumps, spinal meningitis, diarrheal diseases, common respiratory diseases are all serious disabling agents in the military service and on occasion serve to interrupt grievously the training so important in the building of an army.

To the men in the military service then, the control of epidemic diseases is an outstanding problem, and much of the medical officers' activity and usefulness will be found in the field of "Preventive Medicine." This is one of the matters concerning which the medical officer newly accepted in the service may find himself on unaccustomed ground, and here may be a good place to say a word or two on the general duties of medical officers and the experiences that you young men,

most of whom are destined for service in either the Army or Navy, may expect to have in your new environment.

Most doctors are educated, very properly, with the thought that their mission in life is to treat the ill. Their relationship to their patients is very close and very personal. The entire lives of a great majority of physicians are spent in this intimate person-to-person contact with those who call upon their services. It is true that certain elements of the profession have devoted their lives very largely to teaching or to the pursuit of scientific investigative activities which ordinarily remove them from habitual contact with patients; it is also true that within the federal government and within the various state, county, and city governments, there have sprung up departments devoted solely to the public health and to the development and application of preventive measures in relation to disease. But of all the members of the medical profession in America, relatively few are so engaged.

All of the things that the average physician, in common with the rest of the population, lives with, are provided for him by a properly constituted government. His food, water supply, and milk are all produced according to standards laid down by qualified experts and supplied by agents with whom he has only the most remote contact. The areas surrounding his habitation are policed and kept in sanitary condition by apparently invisible agents and his health is protected through the application of quarantine laws with which he has relatively little personal concern. He leads a sheltered and protected life. But when a physician enters the Armed Services, he steps into a new environment.

If stationed at an already established camp, he will find those conveniences necessary to comfortable living that he has been accustomed to in civil life. But more likely than not, he will be called upon to serve with troops in the field and will find himself suddenly thrown into the position of general medical adviser to his commanding officer which means that the commanding officer will look upon this young man not only as a person skilled and expert in every phase of medicine and surgery, but that he is apt also to regard him as an accomplished parasitologist, epidemiologist, sanitary engineer, and whatever other type of specialist the occasion may demand.

To some commanding officers, a doctor is a doctor and they think all these skills should have been acquired in medical school. Needless to say, the degree of success which a young man may attain in his military service will depend largely on his adaptability and enthusiasm; on the rapidity with which he can learn, and the degree in which he can apply the lessons pertaining to his newly presented problems; problems that may have no counterpart in civil life, but which require for their solution the full intelligence and effort of able men.

It is not a simple matter to take a military column into the field and have them come out as healthy as they went in. In the field, a soldier is on his own. He no longer has a city water supply, nor does he have the sanitary appliances to which he has been accustomed in a well-established permanent camp. The very care and preparation of food in the field is a vastly more difficult thing than the same task in a well-screened house in a sanitary community where heat, dust, and insect invaders are not much of a problem. It is in these conditions that the medical officer on a commander's staff becomes a person of very great importance, and only a foolish man would look lightly upon his responsibilities in such a situation. All of these matters, of course, are adequately covered by instructions issued from the War Department and are there for the learning.

The first duty of a medical officer with troops is to learn and apply the simple, but very important, sanitary rules laid down for his guidance. The application of these rules is the responsibility of the commander, but it is the clear duty of the medical officer to keep his commander informed of the steps necessary for the preservation of the health of the troops. No matter how splendid a physician one may be, no matter how expert a therapist; he will have been remiss in his duty as a medical officer if he has failed to put into practice those principles that are known to be helpful in the prevention of the very diseases he may be treating.

There is another thought frequently expressed by young medical men that may be referred to here. That is the belief that in the military service there may be very little chance to practice the profession for which they have been trained. This, they feel, is particularly true in the front line where they may

be called upon to serve as regimental or battalion surgeons. I wish to take this opportunity to disabuse your minds of that fallacy. The thought probably was engendered by the fact that during the period of training, physicians may find themselves, while on duty as regimental or battalion surgeons, engaged in occupations for which they have little liking. But it should always be remembered that the training period is a training period not only for soldiers; it is just as much a time of training for medical officers who are to serve those soldiers. It is here that they must become accustomed to the routine practices of the organization with which they are serving and where they must learn to envision the problems with which they will be confronted when engaged in actual combat: very real and serious problems which have never come within their ken.

The doctor in the battalion aid station must be a strong and resourceful man. He may be the only doctor in his particular post; the only guardian angel between this world and the next for some young wounded soldier. Should he prove inadequate it matters not whether he errs through the failure of the application of ordinary health measures or whether he fails through his lack of knowledge of the steps required to care properly for a wounded man. The fact remains that if he is not a competent man, he does not belong there. The front is no place for a *timid* man. Not only will the doctor have his own fears to overcome, but he will find himself under the necessity of relieving the distress and supporting the morale of the frightened, exhausted, and wounded youngsters given to him as a professional charge. This is no place for the careless or incompetent man. Here the doctor may save lives and know that he is saving them. Certainly he will not work in the excellent conditions that may be found in a well-appointed hospital at the rear, but work he must; and the degree to which he proves himself in his arduous and dangerous and life-saving mission is his measure as a man.

With the advent of this war or, more properly, with the inauguration of our pre-war training program, it became evident that there were many medical problems of a military nature, that demanded immediate and thorough investigation. The thought was entertained that, if a sufficient group of

recognized authorities could be assembled, we might hope for two things: first, a crystallization of thought on presentday medical practices in America; and second, the development of new ideas and new methods calculated to improve our control of war problems.

It was realized that the scope of the proposed studies were greater than could be accomplished within the services themselves, and that aid should be sought of the great body of professional talent in civil life. Accordingly, the Medical Division of the National Research Council was approached with the proposal that they undertake the study of certain problems regarded as important by the medical services of the armed forces. At that time, there were three outstanding problems to be considered: the treatment of shock; the role of sulphonamides in war surgery; and the development of a satisfactory substitute for whole blood transfusion in the battle zone. There were many other matters pertaining to war service on land and sea and in the air that were submitted later, but the three already mentioned were those first taken up for study.

A general Medical Committee and a General Surgical Committee were selected by the Medical Division of the National Research Council and entered upon their deliberations. It rapidly became apparent that special committees would be required for the study of specific problems and appropriate action was taken. This policy was expanded until the whole field of medicine has been rather thoroughly covered by groups of eminent scientists who have been and are now giving freely af their time and intelligence to the solution of the many problems presented.

I cannot speak too earnestly in praise of the work of these groups of distinguished men. There is no doubt that medical thought will be markedly influenced by their contributions and that we shall have a clearer perspective of medicine in America than we ever had before. Furthermore, certain definite benefits are already being enjoyed by our armed forces as the result of these investigations, and we may say without fear of contradiction that many young men are now

alive who would be dead, had it not been for the establishment of life-saving practices determined on by the Army and Navy in collaboration with the National Research Council.

Think of the value we have derived from lyophilized blood plasma. In the last war one of the great bugbears of surgery was the difficulty attendant upon whole blood transfusion. At the front line dressing stations and hospitals every one worked at top speed; bloodless patients at the point of death urgently demanded transfusion, but transfusion is next to impossible in such circumstances. There is no time to type and cross type: donors are scarce, and men already worked to the limit of endurance are not good material from whom to draw blood. Recourse was had at that time to various solutions of a composition deemed suitable for introduction into human veins, but the results were disappointing at best.

What a contrast we now witness! The program of dried plasma procurement, developed by the combined efforts of the Army and Navy and the Red Cross and based on the studies of the appropriate committees of the National Research Council has proven a grand success. Dried plasma, beautifully packaged and ready for instant administration has been made available in large quantities to our troops in the field. The results speak for themselves. From every theater of activity comes the same story—"My life was saved by plasma"; and that story is true; their lives were saved by plasma.

When our troops landed at Casablanca, they operated in the dark of night. The medical facilities available to their service were sharply limited by the military situation, and only the simplest of field medical equipment was available for their support. At midnight, more than four hundred badly burned men were received by the medical authorities. Between midnight and morning all these were handled and adequately cared for. Dried plasma was used liberally—it can be given anywhere; under a shelter tent half; in a sheltered nook; or in the open if necessary—and the result was gratifying beyond expectation. Of the four hundred burned men, only two died; a mortality rate we could not have hoped to approach, had it not been for the use of this life-saving material, developed by medical men as an answer to one of the

questions raised by war. Like experiences are being had by our forces in all our many widely scattered battle fields, and the story of Casablanca has been repeated in varying degree time and time again.

A similar happy result has attended the use of sulphonamides. These also have been made available to our troops everywhere. Each soldier and sailor carries a supply on his person; and all medical installations, from the front to the rear, have been furnished with the various forms in liberal amounts. Their use has been productive of the greatest good. Of gas gangrene we have had very little; infectious processes in wounds are notable for their absence. The saving in man hours represents a significant contribution to the war effort and the saving expressed in terms of human anguish cannot be measured. The sulphonamides are not to be regarded as a "cure-all" and the search for new remedies is being prosecuted with vigor.

Recent observations on the use of penicillin awake the lively hope that here again we have found a miraculous cure. In one of our large military hospitals recently a group of selected men worked with a series of old infected bone cases; battle casualties or men who had sustained trauma in other ways. They had been in hospitals for varying periods up to fourteen months and they looked with despondent eyes into a gloomy future. The use of penicillin was instituted with magical effect—all these cases cleared up in a very short time and the happy result must be ascribed to the remarkable virtues of this newly acquired aid. Once again members of the medical profession, following the lead of British colleagues have, in working on a problem raised by war, conferred a lasting boon on all of humankind.

In all the realm of "Military Medicine," however, there is probably no more interesting subject than that we call "Tropical Medicine," and none at present holds greater interest for those charged with the conduct of medical affairs in our military establishment. Prior to the outbreak of the Spanish-American War, this subject received scant attention in America, and even in the years since then, it has failed to catch the imagination of the great body of medicine in this land to

the degree warranted by its importance. A relatively small group, composed mainly of those officers of the Federal Services who have had service in our overseas stations in the tropics; physicians in charge of the tropical installations of some of our commercial institutions; groups of interested investigators in a number of our educational institutions and officials of certain foundations interested in the problem, have been active in the investigation of tropical diseases and have done splendid work; but to the great majority of American physicians, "Tropical Medicine" remains almost a closed book.

Because of the far-flung action of our armed forces in this war, tropical diseases assume tremendous importance. American soldiers and sailors have penetrated the remote regions of the earth, and there is hardly a torrid land in either hemisphere that has not now its American garrison. And, to the usual peace-time risks of life in the tropics, the difficulties encountered in maintaining health in a period of active military operations are added, vastly complicating the picture. It is clear that, in such conditions, our troops shall certainly be exposed to risks not met in the homeland and the dangerous possibility that a wide dissemination of exotic diseases might easily occur, must always be kept in mind. Among the questions presented to our group of investigators of the National Research Council are many connected with this matter, and it is as a representative of the Council and of the Medical Department of the Army that I wish particularly to bring to your attention the great importance, in our present day world, of "Tropical Medicine."

Let us first seek a definition of "Tropical Diseases." This is not entirely simple, as many diseases so classified are commonly found also in temperate climate and their inclusion in the list of "Tropical Diseases" rests on an appreciation of their increased importance in hot climates.

It has been said "A tropical disease is any disease, as it behaves in a tropical environment. The natural tropical environment is not definable in terms of heat and humidity. It is really a thousand different and complex environments occurring, to be sure, in the warmer parts of the earth, but compounded of special local conditions of climate, social

make-up of the people, social and economic conditions, food materials and especially the arthropod vectors of disease and animal hosts."

This is an excellent and a broad concept, but for our purposes it seems preferable to limit tropical diseases to the following categories:

1. Diseases such as malaria which may be endemic in either tropical or temperate climates, but are more prevalent in the tropics.
2. Filth diseases, such as the dysenteries and cholera, which are more common in tropical countries because of the poor sanitary, hygienic and climatic conditions which prevail in so many regions, but which may cause epidemics when introduced into temperate climates.
3. Diseases which are normally limited to endemic centers in certain tropical regions, but may spread to and cause epidemics in temperate climates as, for example, yellow fever.
4. Diseases which originate in and are limited to tropical regions, such as African sleeping sickness.

The history of "Tropical Medicine" as we know it is not a long one. Apparently, in certain of the ancient Asiatic civilizations something was known of this subject and insects were thought to be vectors of disease. Malaria and its relation to mosquitoes was referred to in ancient Hindu works and the use of nets as a protection against pests was well known. Within the range of accurate historical knowledge, however, these diseases were not much in the limelight until 1878 when Manson, "Father of the modern science of tropical medicine," published his studies regarding insects as vectors of disease. The first treatise in English on tropical disease was that published by Lind in 1768 but the first modern textbook was that of Manson published in 1898. The Liverpool School of Tropical Medicine was established in 1899 and the London School shortly after.

In the United States interest in tropical medicine dates from 1898 when we sent troops to Cuba. There the prevalence of malaria, so much greater than anything our physicians had encountered in America, focused attention on this phase of medicine. There, also, they became acquainted with yellow

fever, the Yellow Jack of the Spanish Main, and it proved a source of much difficulty; but at the same time it afforded an opportunity for Walter Reed and his associates, following up the work of Finlay, to conduct the brilliant series of experiments that resulted in the conquest of that disease.

Gorgas, applying the principles laid down by Reed, cleaned up Havana and later made life endurable in Panama, so rendering feasible the building of the canal in a territory that had defeated all the efforts of the French under DeLesseps. It is no exaggeration to say that the Panama Canal could not have been built, had not Gorgas or someone like him accomplished the thing he did. No wonder the imaginations of young men caught fire! Their professional world was suddenly enlarged and their eyes gazed far away to new horizons established by the late discoveries. Many of the young associates of those older leaders have kept alive, through the ensuing years, their interest in this aspect of medicine. They have made notable contributions in that special field and are now justly looked upon as Fathers of the profession in America.

Ever since the days of the Spanish-American War the Army has maintained laboratories and Medical Research Boards in the Philippine Islands, Puerto Rico, and Panama, and many medical officers connected with these establishments have made noteworthy contributions to scientific medicine, of which the service has every right to be proud. Needless to say, like interest was manifested by our brethren in the Navy and Public Health Service and all three services have derived benefit from their mutual efforts.

However, tropical medicine did not receive adequate attention in the United States until we became involved in the present war which is being fought so largely in tropical areas. With our troops in the Caribbean, Brazil, Africa, India, the Solomons, and other islands of the Southwest Pacific, it is clear that the widespread exposure of our forces to a great diversity of strange diseases causes the whole subject of "Tropical Medicine" to assume immense national importance.

There are many diseases embraced within the term "tropical" and they vary greatly in respect to the menace they present to military forces. Probably, if we should attempt to

list them in order of their potential danger, first place should be given our ancient enemy—malaria. Of all the diseases that afflict mankind, this is probably the most widely spread. It is one of the greatest causes of disability and death and probably does more to wreck the normal structure of human living than any other agent. It is found in most parts of the world, in mild or severe, epidemic, endemic or hyperendemic form. It has been through the ages one of man's most malignant enemies and only within our lifetime have we gained any real information as to its cause and control; and despite the knowledge we now have, malaria still stands, a challenge to the medical profession and a menace to all of humanity. Time and again, even today, it has man reeling back, defeated in his fight with the mosquito to nurse his ailments for weeks or months before resuming his accustomed occupations.

Yellow fever, although not commonly considered a very great danger since the discoveries of Reed, must still be reckoned with in most respectful terms. Although our efforts toward eradication were attended with great success in Cuba; although we have during the past generation or more, developed a great sense of security concerning the possibility of yellow fever invading our own shores or dependencies, we have, in the past few years, received a violent shock from the knowledge that there is a type of yellow fever prevalent in the South American and African jungles, maintained chiefly by susceptible forest animals and that the possibility of transmission to our territories must be ever in our minds.

The various dysenteries, whether amoebic or bacillary, assume a place of prominence in any consideration of this subject. In the early days of our occupation of the Philippines these diseases caused great trouble. Little was known of them; the Army was composed largely of recently organized units which operated in the most difficult of circumstances in a lush and unsanitated area. The disease incidence was high and the lesson was sharply etched on our consciousness. Even today the same danger exists. It is exceedingly difficult to protect men properly in the field against diseases of this type and filth; flies and fingers require attention just

as certainly as they did in the days of our early tropical ventures.

Asiatic cholera is a disease which has unpleasantly and disastrously visited our troops and, indeed, our own land on more than one occasion. We had it in the Philippines; Scott's Army had it in Mexico; and your texts have informed you of the outbreaks in America. Although essentially an Asiatic disease, it can become a menace anywhere and, unfortunately, our protection is not good.

As one who has had dengue, I can assure you that it is a disease not to be laughed at in one's lightest moments. It can appear anywhere the Aedes Aegypti mosquito thrives; it is exceedingly common in many tropical regions and, of course, has on occasions visited the American mainland. Happly this is not a fatal disease, but it can cause great disability and must be counted as a pronounced military hazard. Those of you who are so fortunate as to be given service in the tropics will almost certainly encounter it. It may even be one of your chief problems.

Hookworm and other helminth infestations are exceedingly common in the tropics and there also you may see the enteric fevers in a form not usually seen in our land. Because of the increased virulence they sometimes exhibit, and because of the difficulties attending their sanitary control in the torrid zone, the enteric diseases may properly be included in the term "tropical diseases." We shall refer to them later.

Typhus is notoriously a disease of armies—and while not essentially a tropical disease, it thrives wherever sanitation is poor, men are crowded together and protection from vectors is faulty. When we remember that the rickettsial diseases, of which this is one, may be transmitted by lice, fleas, mites and ticks, the wide range of possible infection becomes evident—and all these pests are found in the tropics.

Plague, leishmaniasis, schistosomiasis, trypanosomiasis, yaws, leprosy, smallpox and fungus infections all properly fall within the scope of these remarks and all present arresting problems.

Nutritional disorders are apt to be prevalent in the torrid zones. The populace, more likely than not, is poor, ignorant and improperly fed. Avitaminoses are common; beri-beri in particular has been a serious problem in the past and can easily become so again unless careful watch is kept upon the diet. Scurvy is an old acquaintance and is to be feared. The danger to our own troops lies in the fact that, in widespread warfare, it is exceedingly difficult to maintain an unbroken flow of necessary and accustomed articles of diet; and in time of real need, our men would be at a disadvantage in that they have not the same knowledge regarding edible articles locally available as have the natives.

There are disease conditions in hot climates that may be called "climatic diseases." These embrace "heat-exhaustion" and "heat-pyrexia," with both of which you are familiar, and the so-called tropical neurasthenia. It is a fact that many white persons do not prosper in the torrid zone and after a period of residence therein develop neurasthenias of such character that return to the colder climate becomes imperative. Others of us, however, do well enough and, for many persons, life in the tropics is a delightful experience. It is quite likely, however, that you will, during your tropical sojourn, encounter such cases and you should be prepared to care for them.

We have reviewed, very briefly, some of the conditions not frequently encountered in our homeland, but which are widespread in the tropics. They constitute a common feature of medical practice in such areas and they offer a fascinating field for medical investigation. Moreover, they hold for us a consuming interest because of the strong possibility that they may be introduced into our own land by men returning from overseas.

The very real danger of the introduction of these foreign maladies is before our eyes this very moment. When we consider the enormous traffic now going on between America and tropical countries, by sea and by air, the difficulties of maintaining an effective quarantine service become readily apparent. Carelessness at a critical moment might easily

spell disaster, and constant vigilance is necessary for the protection of the health of the nation. An example may serve to fix this thought more clearly in your minds. If you should draw a line running north between New Zealand and Australia, encircle New Caledonia passing from west to north and then down the east coast; make a reverse loop to the north, embracing the New Hebrides; extend the line along the north coast of New Guinea and then north to the east of the Philippines, thence east to embrace the Hawaiian group and then south roughly following the west coast of America, you will have divided the Pacific Ocean into two regions. All those lands to the south of the line have no Anopheles mosquitoes and consequently no indigenous malaria, while the lands north of the lines are heavily infected. Now see New Caledonia in the west—Anopheles free; the Fiji group to the east also free, while in the loop between them the New Hebrides lie—a hotbed of malaria and to the north and west we see New Guinea and the Solomons, also notorious malarial areas.

It is not hard to imagine what havoc could be created in the islands now free of malaria should infected Anopheles be introduced by ship or plane from the dangerous areas nearby. The wonder is that this has not already occurred. We accept the fact as we find it and are making every effort to maintain conditions as they are. You can realize that with war being fought on a global basis and military movements being executed on an oceanic scale, this situation has profound epidemiological significance and effective quarantine becomes a matter of peculiar importance. Problems of this nature occupy the thought of those charged with the maintenance of health in our armed forces and problems of like nature will engage your attention while in the military service; perhaps not in the same grand design but in a degree suited to your official position in the military medical hierarchy.

The control of diseases then, including tropical disorders, is of paramount importance in the military service, and we can do a lot toward their control. This may be done by adherence

to prescribed rules for sanitation and hygiene, referred to before, and in certain conditions we have, by the use of specific preventive measures, worked enormous improvement.

Let us think of the enteric diseases. These are chosen because they are the classical type of military disease. All armies, ours and those of foreign nations, suffered for centuries from them and they have, on occasions, been so prevalent as to practically immobilize armed forces. When I entered the service in 1907, typhoid was one of our common causes of disability, and it was customary to set aside special wards in army hospitals for the treatment of such cases. At that time there was much typhoid in the country; there was a lot of it in the Army and all physicians were familiar with it. In 1909 the Army adopted, as a voluntary measure, inoculation against the disease and a year or two later made the practice compulsory for all military personnel. In 1910, at a regimental post in the southern Philippine Islands, I came in contact with the tail end of an epidemic of typhoid fever. The regiment stationed there had more than 100 cases and the mortality rate was high. That regiment was moved out, the post was cleaned up and another regiment moved in. At the same time the use of the typhoid prophylactic became firmly established in that region. From that day to this, I have never seen a case of typhoid fever in any person in the military establishment. There have been cases but neither I nor many other medical officers have seen them. In the Spanish-American War our record in this respect was shameful. In the First World War it was good and in this present war the incidence of the enteric diseases is so low that the rates are infinitesimal. We may say with a good deal of confidence that, for the armies of the United States, these diseases have lost their danger.

The control of yellow fever implies control of the Aedes mosquito and in those regions where we had formerly been menaced by it our control methods have been painstaking and successful. But suppose some infected Aedes should be carried to the islands of the Pacific which have never experienced yellow fever, but which are heavily infested with Aedes. The thought is not pleasant. What we noted about the imaginary line across the Pacific and Anopheles mosquitoes has nothing

to do with Aedes Egypti. All those Anopheles free lands are heavily infested with Aedes and it is not difficult to imagine the disaster that might follow should some inexperienced or careless person cause a break in quarantine that would permit infection of the populace in the now yellow-fever-free regions.

You may be interested to know of the success that has attended our efforts in the control of malaria in the Army in this country during the present war. In 1939 we laid plans for the eradication of this disease in our southern camps. An agreement was arrived at whereby the Public Health Service undertook to sanitate the extra-cantonment areas while the Army concentrated on the military reservations. Much money was spent, but well spent, and great success attended our efforts. The malaria rates in the Army in the United States for 1940 were reduced to the lowest point since the Revolutionary War: 1.7 per thousand per annum; and the following year a further reduction was attained, bringing the rate to .65 per thousand per annum, a figure previously thought impossible of attainment by those most widely skilled in that particular field.

These diseases are selected particularly because they are shining examples of the good that may be accomplished by earnest men diligently applying the simple rules of preventive medicine we have mentioned before. It must be borne in mind that preventive medicine will be at the very root and base of your work as medical officers in the military service.

For the protection of our troops we resort, wherever possible, to the use of specific measures based on proper scientific thought. The enormous good that has been accomplished by vaccination against smallpox is known to all; prophylaxis against typhoid has worked enormous good; and the elimination of tetanus from our Army by the use of tetanus toxoid is a notable accomplishment of this war. For the protection of our troops, there has been developed a vaccine against yellow fever which is administered to all troops departing for tropical countries. Our allies take the same precaution and are very insistent on its observance. We have faith that this measure is definitely protective and hope to escape trouble

from this source. We also immunize against cholera and typhus and hope for a satisfactory degree of protection.

But, remember our chief dependence for the control of tropical diseases must be placed on the rigid observance of sanitary and hygienic rules. Our water and food must be protected; disposal of wastes must be properly accomplished; the diet must be adequate with special attention to the dangers of avitaminosis; and protection must be secured against recognized vectors of disease by the use of drainage and oiling of swampy places when feasible; by the use of nets, screens and insect repellents and by appropriate drug prophylaxis when indicated.

I have not attempted a discussion of the clinical features or treatment of any specific disease. Your instructors are better qualified to do that, and, I am sure, you have already been fully informed, or shall be shortly, on the care of the diseases we have in mind. I have merely tried to emphasize the overwhelming importance from the military viewpoint of disease prevention, particularly the control of tropical diseases. In a larger degree than ever before this subject must command our attention. From my observation in Africa and South America and from reports given me from our Pacific bases, I know that American medicine has a tremendous problem ahead of it. If, after this war, we are to engage in widespread rehabilitation enterprises, preventive medicine will be an outstanding feature of the program, and preventive medicine will mean largely, campaigns for the control or the eradication of the disabling conditions now so common in the tropics. I am sure that for a long time to come the American profession will be largely engaged in this phase of medicine and that a large part of the world will look to us for leadership and assistance. The prospect is full of romance and discoveries of far-reaching importance will doubtless be made; discoveries in which the young men now coming up in medicine will be privileged to participate. The battle must be fought on the three fronts of prevention, therapy, and research, each supporting the others. You are the men who must do it, you and your fellows, for in your hands lies the future of American medicine.

DETERGENTS AS PHOPHYLACTICS AGAINST VESICANT EXPOSURE*

DAVID F. MARSH AND CHAUNCEY D. LEAKE

Soap and water are highly effective for removing from the intact skin such vesicants as lewisite, mustard gas, or the nitrogen mustards, if used within three to eight minutes after exposure. If the delay is longer than this, the vesicant penetrates deeply into skin surface lipoid material, particularly concentrated around hairs, where it is not easily removed either by soap and water or by mild oxidizing agents such as hypochlorite solutions. Various detergents have been investigated in order to determine if any might be found which might allow more time before treatment would have to be instituted.

Aliphatic mono-amines with twelve to eighteen carbon atoms will preferentially absorb mustards or lewisite in comparison to skin lipoid, but the complete removal of the amine from the skin is difficult and any remaining material may cause damage.

Sodium alkylsulfonates and sodium dialkylsulfosuccinates are equal in activity to soap although their emulsions of mustard gas and water are unstable. The alkaline detergents and partial fatty acid esters of sorbitan are unsatisfactory, since they are poorly water soluble. The acitergeols and polyoxyalkylene derivatives of sorbitan monolaurate are effective detergents in removing mustard gas, even when dissolved in lipoid, but the allowable time delay is only ten to twelve minutes.

It is clear that soaps and water remain the most effective and *readily obtainable* detergents for prompt use in self aid when exposure to war gas vesicants is suspected.

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RENAL PARENCHYMA, BLOOD PLASMA, AND INTERSTITIAL TISSUES IN THE MAINTENANCE OF BODY FLUID EQUILIBRIUM AS PORTRAYED IN THE DIURETIC EFFECTS OF XANTHINES AND MERCURIALS*

GEORGE R. HERRMANN AND GEORGE M. DECHERD, JR.

We have been investigating edema since 1930 and have made extensive studies before, during, and after the periods of effectiveness of powerful diuretic agents, in order to get data on the mechanism of edema mobilization and dissipation and on the mode of action of diuretic drugs (Herrmann and Decherd, 1937, 1940).

Most disturbances of circulatory equilibrium, especially those presenting venous congestion, are accompanied by significant shifts in body fluids. As water accumulates in the interstices of the tissues, the load on the heart apparently increases, congestive heart failure progresses, and the patient becomes more and more waterlogged with edema. The usual steps taken to interrupt and reverse such a situation depend upon measures that facilitate the return of the excess of interstitial tissue fluid to the blood stream for transport to the kidney and excretion in the urine.

The circulatory system maintains a remarkable constancy (homeostasis) in the general bodily milieu. This probably requires for its accomplishment only relatively minute changes in the physical and chemical constitution of the blood plasma, tissue fluids, and glomerular filtrate, and in renal metabolism. Nonnenbruch (1933–1937) published an extensive review of the whole subject. We have felt from our studies that explanations of edema formation and dissipation are intimately associated with plasma electrolyte balance. Shifts in electrolytes and colloids under normal conditions are so slight that, in most efforts to follow these shifts, our present micro-methods

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have been found to be inadequate. We have hopes that improved methods will be forthcoming.

Increase of urinary output, by means other than the administration of large quantities of fluid or water by mouth, subcutaneously, or intravenously, produces proportionately great shifts. These we have considered possibly great enough to furnish some clues as to the roles played by the salts, water, and proteins in the maintenance of water balance, and in the excretion of urine, and might well contribute to the elucidation of the fundamental problems of renal function (Herrmann and Decherd, 1937, 1940). The provoking of a profuse diuresis by the use of mercurials, xanthine preparations, or digitalis glucosides introduced intravenously are a part of good medical treatment of dropsical patients. Studies during such procedures have thrown some light on the mechanisms by which fluids are mobilized and excreted. Data from patients with congestive heart failure have established the effects of the drugs on tissues that are not entirely normal. Such tissues may definitely function differently under the stress of high diuresis. However, the results may be such that even under pathological conditions helpful and reliable information can be gleaned, and this may serve as an approach to the fundamental problems.

At the same time the mechanism of diuresis by various therapeutic procedures in edematous patients may be clarified and simplified. In spite of the great amount of laboratory research and clinical investigative work that has been done, there still is need for prosecution of clinical experiments, for elucidation of the mechanisms of the actions of the various diuretics.

Our studies during the past decade have been toward these ends. In the earlier years we concentrated our efforts on the attempt to determine the relation of the plasma electrolytes and proteins to the presence and absence of edema. Then, we studied the possible direct initial effect of xanthines and mercurials and the point of primary action in the kidneys. We are now trying to follow the possible indirect or subsequent or side effects, and the secondary mechanisms that are most likely thrown into play during diuresis. Our studies have yielded much practical information and some interesting data on the theoretical aspects of fluid balance, mobilization and

excretion, and of electrolyte or salt exchange and plasma protein levels. A tremendous amount of experimental investigation and clinical research has been vigorously pursued in the hope of establishing a comprehensive theory of the mechanisms involved in salt and water balance.

The importance of major circulatory shifts, systemic arterial blood pressure, capillary pressure, venous pressure, glomerular changes, and blood flow in the kidneys, functioning as the chief excretory organs, have long been studied. Blood volume and the blood constituents are generally recognized as significant factors. Extensive studies have been consummated on the genesis and significance of subcutaneous edema, water retention, and sodium chloride or electrolyte concentration in the depots of tissue fluid storage. The role of blood plasma albumin and globulin levels and ratios and consequent colloid osmotic pressure changes have been noted. The permeability of the capillary walls; intracapillary blood pressure; the osmotic pressure and fluid pressure in tissue interstices; the passage of fluids and electrolytes through the tissues; the relinquishment of stored water and salts by the tissues; and the role of the liver in water metabolism have been studied. The effect of xanthines and mercurials on electrolyte clearances and plasma protein balances, and the blood plasma volume changes are some of the approaches to the investigation of these problems that we have attacked.

The Anatomical Kidney Unit and Its Physiologic Relationships

Into the uppermost end of each of one and one-half million or more nephron tubules, afferent arterioles coil into an expansive network, invaginating the delicate epithelial layer of Bowman's capsule and forming the broad filtration surface of the glomerular tuft. The surface area of the glomerular capillary bed is calculated to be about one million square millimeters (Krogh, 1922). Of similar importance is the anatomic fact that the efferent arteriole from each glomerulus follows the renal unit pattern and breaks up into a capillary bed in intimate contact with the continuation of the nephron, the epithelium-lined proximal and distal convoluted tubules. Some

few direct vessels to the tubular plexuses have been found by MacNider (1931) to be present in diseased kidneys. Through these units the blood is brought into contact with the epithelial cells of a semipermeable membrane. The cardiovascular-renal system tends to maintain a remarkable constancy in the general bodily electrolyte and fluid balance (Barcroft and Straub, 1910). Some water and some salts are eliminated to maintain the osmotic balance and the acid-base equilibrium, and the nitrogenous waste products are removed from the blood and discharged from the body (Cushny, 1925). Glomerular filtration and active tubular reabsorption are now generally considered to be most significant factors in the maintenance of normal water and salt exchange (Richards, 1929). A consideration of the anatomy and particularly of the blood supply of the nephron makes it obvious that, when the glomerulus fails, the tubule closely follows suit, and complete dysfunction promptly results.

Theories of Kidney Function

Theories of kidney function have undergone changes since 1842, when Bowman postulated that water was secreted by the glomerular tuft, and that the uriniferous products were secreted by the tubular epithelium, added to the water, and collected by the ducts as urine. Ludwig (1844) contended that production of urine consisted in the mechanical filtration of a protein-free solution through the glomerular membrane and reabsorption by diffusion of some salts and water. Heidenhain (1874) revived the vitalistic secretion theory, adding that, in addition to water, chlorides were selected and secreted by the glomerular epithelium, while organic nitrogenous waste substances and dyes were secreted by the lining cells of the tubules.

Barcroft and Straub (1910) replaced blood plasma with Ringer's solution, retaining the red blood cells to insure oxygenation, and perfused this through intact kidneys with the production of an augmented urine flow without a rise in blood pressure. Knowlton (1911) found that addition of a colloid, such as gum acacia, to the plasmapheresed erythrocyte solution, or to a hypertonic glucose or saline solution introduced

intravenously, prevented a diuresis. Cohnheim in 1912 (quoted by Eppinger, 1917) demonstrated that glomerular membrane cells had no selective secretory function, for if the body was deprived of salt, sodium chloride still appeared in the glomerular filtrate and had to be conserved by tubular reabsorption.

Cushny (1917) conceived of a purely physical glomerular ultrafiltration and of a non-vital selective diffusion tubular reabsorption. The protein-free glomerular filtrate was postulated to be identical in electrolyte composition with plasma and contained water, salts, particularly sodium chloride, and also urea, uric acid, amino-acids, creatinine, creatine, and glucose. The tubular epithelium permitted diffusion through it of those threshold substances useful in body economy and in maintaining water balance; these were reclaimed by tubular reabsorption of all of the glucose, amino-acids, and some of the salts and water. Cushny vehemently opposed every suggestion of tubular excretion.

Starling, as early as 1899, demonstrated the osmotic pressure of blood colloids to be 30 mm. Hg. by putting a filtrate of blood plasma through a gelatin filter. With Verney (1924) he added to Cushny's conception the theory of tubular secretion of dyes and urea, later confirmed by Hober and Marshall (1934). Richards, between 1910 and 1924, and his students between 1924 and 1938, by capsular and tubular microcatheterization proved the glomerular filtration theory of Cushny; they demonstrated the actual specific reabsorption of various substances by the proximal, and others by the distal, convoluted tubules.

Rehberg (1926) agreed to the glomerular filtration of a dilute dialysate of the blood plasma, but he too would endow the tubular epithelium with power of discrimination in reabsorption. He considered a substance threshold only if the percentage in reabsorbed fluid, under normal conditions, was higher than in the blood. Creatinine alone he accepted as entirely a waste, totally nonthreshold substance, with phosphates, calcium, and sulfates as possibly so.

Homer W. Smith (1919) and associates (1925 to 1940), particularly Shannon (1935), demonstrated the fact that creatinine was not an absolute index of glomerular filtration but

was apparently added in part by the tubular epithelial cells. In addition, dyes, such as phenol red, and organic iodine preparations, such as hippuran, diodrast, and skiodan, have been proved to be added by tubular excretion. D-xylose, raffinose, sucrose, inulin, and mannitol were found to be the best indicators of glomerular filtration.

The current theory generally accepted considers the glomerular fluid to be a simple filtrate of the blood plasma, minus the serum proteins and fats, similar to that passed through a gelatin filter from a colloid-salt solution. The human tubular epithelium then enters into active selective reabsorption of water and substances to be conserved, and probably adds to the concentrate creatinine, dyes, and organic iodide compounds.

Any consideration of the functions of the kidney must be concerned with the multiple extrarenal factors, blood plasma, electrolytes, nitrogenous waste, colloids, and intravascular and tissue pressures. Excretion of urine has been stopped when pressure in the ureter was raised to about 90 mm. Hg., with the blood pressure of 130 mm. Hg. giving a differential pressure of about 40 mm. Hg. Dilution of the colloids in the plasma by adding urea, ammonium or potassium chloride or nitrate by mouth, or hypertonic glucose or inert sugar and salt solutions by vein, draws water and salt from the tissues, lowers the pressure of the colloids, and inaugurates increased filtration and urine output. Changes in the circulation, such as congestive failure with anoxemia, and acute shock, have been observed to produce oliguria or anuria. Water balance disturbances by dehydration by diarrhea, vomiting, or sweating decrease the urinary output considerably.

Significant Chemical Constituents of Blood and Urine

The blood plasma and dilute and concentrated urine content of the more common substances are of interest. For instance, the water percentages for blood plasma are 90 to 93 per cent as contrasted with 93 and 95 per cent for dilute and concentrated urine; the chlorides 0.370 gm. per cent as contrasted with about 0.500 and 2 gm. per cent; urea 0.030 gm. per cent as contrasted with 2 and 6 gm. per cent; uric acid 0.004 gm. per cent as contrasted with 0.050 and 0.129 gm.

per cent; creatinine 0.001 gm. per cent as contrasted with 0.075 and 0.750 gm. per cent. Creatinine and creatine are related to the muscular development and are constant for the individual; hence only the percentages shift. Ammonia is increased with increased acidity of the urine, and a high protein diet increases urine acidity, ammonia, and unidentifiable rest-nitrogen. Ammonia increases also with nitrogen intake. Undetermined rest-nitrogen amounts to between 2 and 12 per cent of the total. A high protein intake of 100 gm. per day yields a nitrogen output of 14 to 16 gm. of which 80 to 90 per cent is urea. A low protein diet of 30 to 40 gm. yields 4 gm., of which 60 to 70 per cent is urea.

The inorganic salt metabolism and acid-base equilibrium concern chiefly sodium and chloride. Potassium, calcium, magnesium, carbonic acid, phosphates, and sulfates are of some significance, while copper, zinc, and foreign substances, such as thiosulfates and sulfocyanates, play very minor roles. The sulfates, though likewise unimportant, are found in inorganic and in ethereal organic acids, protein amino acid, cystine, cysteine, and as neutral sulfur. According to Gamble (1939) the bases in milliequivalents per liter as Na 142, K 5, Ca 5, and Mg 3, a total of 155, are balanced against the acid total of 155 made up of HCO_3 27, Cl 103, HPO_4 2, SO_4 1, organic acids 6, and protein 16 milliequivalents.

The great reservoir of body water is the intracellular fluid; it represents about 50 per cent of the body weight. The intracellular fluid contains potassium as its chief basic ion and organic phosphate as its chief acid ion. The interstitial fluid makes up only 15 per cent of the body weight and differs in that sodium is its chief basic ion and chloride constitutes its chief acid ion. The interstitial fluid is more easily mobilized. The blood plasma amounts to only five per cent of the body weight, but it is most active in water metabolism and presents evidence of very delicately controlled acid-base and electrolyte balances.

The kidney has two important functions to perform. It must regulate the salt and water exchanges so as to keep the osmotic pressure of the blood constant, in addition to removal of the non-volatile, nonprotein nitrogenous waste

products of protein metabolism. Normal circulating blood plasma exhibits a delicate balance of electrolytes and colloids in equilibrium with those of the fluids of the interstitial tissues. A balance is normally established between intravascular blood pressure and osmotic pressure of blood colloids.

Blood plasma proteins are retained by the normal semipermeable glomerular membrane, while water and electrolytes filter through into Bowman's capsule in equimolar concentrations. The dilute glomerular filtrate is concentrated a hundred-fold as the water, glucose, and some of the other constituents pass back through the epithelium of the convoluted tubules and the loop of Henle and through the walls of the distal capillary bed into the circulation. The increase in circulating blood plasma volume *per se* does not increase the urinary outflow.

Regulation of the kidney function by changes in the make-up of the blood has been demonstrated following electrolyte and colloid shifts. These slight changes in the blood plasma have been found promptly balanced by greater shifts of electrolytes and water from the interstitial tissues. Normal urinary constituents and other acid salts have been used to cause an increase of the plasma electrolytes to upset the electrolyte equilibrium and inaugurate an inflow from the tissues which in turn precipitates an increased urinary output.

Circulatory Factors in Kidney Function

Normal kidney units require an active adequate circulation as well as an expanded capillary bed in order to provide a sufficient filtration area. At the same time, however, there is a good deal of evidence that something more than physical processes are going on in the kidneys. Work is actually accomplished. Oxygen is utilized in renal metabolism, hippuric acid is synthesized from glycine and benzoic acid, and ammonia is formed. Utilization of oxygen by the isolated kidney in considerable amount, with carbon dioxide formation, has been demonstrated experimentally in the blood and by microanalysis. Oxygen consumption by the isolated kidney is considerably greater under conditions of increased activity. This has not been demonstrated in human beings. However,

the ingestion of large amounts of fluid by normal individuals has been found by continental physiologists to produce a diuresis without a demonstrable increase in the general oxygen consumption. The forced fluid intake results in an increase in the minute output of the heart by an increase in the stroke volume with actually a decrease in the pulse rate and in the venous pressure and only a slight rise in the arterial blood pressure.

The kidneys, by virtue of the tremendous blood flow through them, constitute one of the four most vital capillary systems of the body. The vascular and renal systems are physiologically interdependent, the kidneys perhaps playing the major role, but requiring, nevertheless, in order to be effective 25 per cent of the normal total blood flow. In the kidney, constriction of the afferent or the efferent arterioles of the glomerular tufts is possible and may affect the function oppositely.

In congestive failure of the circulation, venous stasis increases the outflow, from the increased blood plasma volume of water and salt, into the tissue interstices. There are several other factors at work in causing oliguria besides retention of salt and water in the tissues. A decreased filtration through the glomerulus may result from intraglomerular cappilary pressure decrease, with the usual fall in the systolic arterial pressure, in spite of the increased blood volume and the usually decreased blood colloid osmotic pressure. Tubular reabsorption remaining normal, the water content of the circulating blood increases. The general circulation may be improved by cardiac tonics and other measures, and diuresis results. The local circulation through the glomerular capillary tuft of the kidney may be influenced by drugs to dilate proximally or distally, or to contract likewise, and by such changes of blood flow affect filtration or reabsorption.

Diuretic drugs, by changing the glomerular blood flow, upset the electrolyte balance of the blood plasma. This change seems to command the rapid mobilization of salts and, secondarily, of fluids, from the tissues, and the transport of both to the kidney for excretion in a diuresis. The accumulated abnormal amounts of water and salts in the tissue interstices

and the edema of the failing circulation, are mobilized, thus evidently increasing the urinary output indirectly.

The primary regulation of kidney function by circulatory shifts *per se* has been repeatedly denied. Experimentally diuresis has been inaugurated and suspended with the maintenance of a constant blood pressure level. A pulse pressure change in the circulation may be the chief influence in modifying kidney function. Nonnenbruch concluded that the filtration theory of renal function has exaggerated the significance of circulatory changes and the effect of arterial, capillary, and venous blood pressures on salt and water balance. He admitted that the abnormally high susceptibility to stimulation of the renal arterioles might result in contraction or dilatation, but this thesis has not been proved experimentally as a significant factor in urine production. Many studies on the capillary bed in general physiology have revealed striking independence of the capillary flow and of the size and of the tension in the arterioles. The status of the capillary bed as a whole may be significant. At the extreme limits the capillary flow must undoubtedly affect urinary output.

The effect of heat on the kidney, and particularly on its blood flow, has interested clinicians who have accomplished an increase in urinary output with diathermy in patients with anuria. Denervated kidneys have not responded in the same way. Cold, however, did not produce exactly the opposite effects. It seems that the effects are the results of reflexes. These reflexes in case of heat, it is stated, do not result in increased circulation, and yet there was increased urinary flow. In acute nephritis, the high blood pressure is associated with oliguria, and lower levels, with increased urinary flow.

There may be quite a group of other variable factors effective in changing urinary flow, yet diuresis is not independent of an increased renal blood flow; in fact, it needs it. The two go hand in hand; but we may have increased flow without diuresis, and diuresis without increased blood flow may be the result of the increased excretion of salts and water or of decreased tubular reabsorption. Chemical influences may cause a dilatation of the afferent or efferent glomerular arteriole and allow increased circulation; or a constriction may

have the opposite effect. Frequently a prompt increase in renal blood flow is the primary response in the kidney's complicated activity.

Interstitial Tissues

In order to explain the relatively a-metabolic changes represented in water and salt shifts, the vastness of the capillary bed in the interstitial tissues all over the body, as well as in the kidney, must be taken into account. Krogh (1922) estimated a capillary system of 10,000 meters in length and 63,000 square meters in surface area. In such a system the rapid exchange back and forth between the important electrolytes and fluids in the intercellular tissue spaces and the blood plasma may easily be visualized as taking place rapidly as purely physical phenomena. Anoxemia or injury of the capillary wall of a significant segment of the system would cause edema formation. It has been estimated that the water content of the plasma changes about a hundred times a day, and that the whole water mass circulates throughout the body and makes a complete circuit probably six times each day.

The interstitial tissues and connective tissues contain a very large extra-cellular reservoir or depot for the body fluids, salts, and other constituents. The capillary walls are single-cell thick semipermeable membranes through which water and electrolytes seem to pass back and forth freely. The colloid serum proteins, on the other hand, are held back almost completely in the normal glomerulus. It is evident, however, from data on blood plasma protein shifts during diuresis and following hydremic plethora, that in some areas the capillary walls permit the passage of serum proteins out of the tissue spaces into the blood plasma. The general rich capillary distribution makes possible the maintenance of equilibrium with relatively prompt return to the constancy of the blood plasma volume and its constituents.

Nervous and Hormone Factors

Angioneurotic edema, allergic effusion, diabetes insipidus and its control with pitressin, myxedema, and desoxycorticosterone plethora are evidences that the nervous system and the

glands of internal secretion play important, albeit largely unexplained, roles in water metabolism. Studies of the antidiuretic effect of pitressin in patients with diabetes insipidus indicate that the hormone pitressin acts directly on the kidney in some still rather obscure way which is not related to the blood flow. At the same time there is, following injection of pitressin, much interstitial tissue effect which cannot as yet be elucidated.

Diabetes insipidus may result from injury to the posterior lobe of the pituitary or the hypothalamic region and tissues of the midbrain or the diencephalon. The posterior pituitary body is apparently linked physiologically with the great nuclear nerve origins at the vasomotor and other significant vegetative centers in the diencephalon. These centers are all susceptible to nervous, humoral, and hormonal stimulation. There is no proof that there is a decrease in pitressin in the blood stream to cause the thirst and diuresis of diabetes insipidus. The antidiuretic effect of pitressin persists in spite of the continued ingestion of large volumes of water and the addition of vasodilating drugs. The reported findings of abnormalities in the cells of the anterior lobe of the pituitary following diuresis and the apparent dehydrating effect of thyroxin have suggested the possibility of a disorder of the anterior pituitary thyrotropic hormone in myxedema.

The relatively recently discovered sodium, potassium, and plasma water balance disturbances in Addison's disease indicate that the adrenal cortical hormone is a significant factor in salt and water metabolism (Harrop, 1937; Thorne, 1942). The striking water storage effect of the synthetic hormone desoxycorticosterone is a fact that invites further investigations.

It is thus evident that the problems of water balance and water metabolism, edema formation, and diuresis have been extensively studied from almost every conceivable point of view. Water intake, urinary output, blood plasma volume, and tissue fluid pressures have been shown to be most significantly concerned. In absolute water metabolism a delicate weighing is necessary to take into account the water that is lost in insensible perspiration, in exhaled air, and in the stools.

Shifts in the individuals' body weight as determined by ordinary balances may record grossly the dissipation of the tissue edema, but the measured output of fluids through the kidney is the chief clinical index we have used. Normally the whole process from absorption of fluid through the gut and transport through the tissues and in the blood plasma to the kidney takes place without significant changes in the blood circulation time, volume, or pressures.

Excerpts from Our Studies

The senior author inaugurated studies on water metabolism in 1930 by determination of the blood serum calcium, inorganic phosphorus, and plasma protein levels in patients with cardiac edema, and after dissipating the edema by diuresis. It was found that there was good correlation between the reductions in the total blood plasma proteins, serum albumin and globulin and the presence of edema (Herrmann, 1930). A further study of a series of patients with various types of heart disease presenting dependant edema of congestive failure, and later without congestive failure and edema, showed a definite lowering of the total plasma proteins, particularly of the serum albumin, and a slight compensatory rise in the serum globulin in the presence of edema. See Table I (Herrmann, 1931). It was further established that rest, cardiac tonic, and diuretics, while mobilizing edema, were accompanied by relatively slight shifts in the plasma proteins in comparison with the striking clinical improvement that the patient exhibited. It was suggested that the delayed blood chemical recovery was related to the residual effects of previous hepatic edema and anoxia incident to congestive failure. Recovery with a sharp drop in blood protein levels after venesection was expected, but it was surprising to find decreased levels of blood proteins during brisk diuresis. It was postulated that during diuresis hydremia and dilution of the blood constituents prevailed.

Studies of the renal function during the dissipation of cardiac edema by determining the clearances of urea, sodium chloride, particularly creatinine clearance, according to the method of Rehberg (1926), yielded significant data. Clearances determined before and during the action of various diuretics, as

TABLE I

Group.	Serum Albumin.		Serum globulin.		Total protein.		A/G ratio.	
	Average of lowest.	Average of highest.	Average of lowest.	Average of highest.	Average of lowest.	Average of highest.	Average of lowest.	Average of highest.
Hypertension, arteriosclerotic (chr. neph.):								
With edema (40 cases)	3.38	3.82	2.13	2.64	5.51	6.11	1.55	1.98
	—29%	—20%	+12%	+39%	—17%	—8.8%	—38%	—21%
Without edema (25 cases)	4.03	4.10	2.41	2.51	6.51	6.66	1.73	1.79
	—16%	—15%	+27%	+32%	—2.8%	—0.6%	—31%	—30%
Arteriosclerosis with auricular fibrillation:								
With edema (5 cases)	3.11	3.53	1.99	2.22	5.19	5.67	1.60	1.81
	—35%	—26%	+4.7%	+16.8%	—22.5%	—15%	—36%	—27.6%
Without edema (8 cases)	4.27	4.61	2.69	3.08	6.92	7.54	1.49	1.91
	—11%	—4%	+42%	+62%	+3.3%	+13%	—40.4%	—24%
Syphilitic aortisis:								
With edema (9 cases)	3.04	3.46	2.15	2.83	5.26	6.26	1.29	1.68
	—36.7%	—28%	+13%	+49%	—22%	—6.6%	—48%	—33%
Without edema (3 cases)	3.44	3.75	2.76	2.77	6.20	6.52	1.36	1.49
	—28%	—22%	+45%	+46%	—7.5%	—2.7%	—46%	—40%
Syphilitic aortic regurgitation:								
With edema (10 cases)	3.16	3.48	2.60	2.15	5.36	6.00	1.34	1.58
	—34%	—28%	+37%	+13%	—20%	—10.4%	—46.4%	—36.8%
Without edema (4 cases)	3.82	4.30	2.29	2.48	6.17	6.72	1.57	1.87
	—20%	—10%	+21%	+31%	—7.9%	+0.3%	—37%	—25%
Rheumatic mitral stenosis:								
With edema (3 cases)	3.29	3.52	2.12	2.58	5.44	6.67	1.41	1.64
	—31%	—27%	+12%	+35.8%	—19%	—0.4%	—44%	—34%
Without edema (6 cases)	3.86	4.46	2.21	2.44	6.10	6.87	1.71	1.98
	—20%	—7%	+16%	+28%	—9%	+25%	—31.6%	—21%
Normal average	4.80		1.90		6.70		2.50	
Range	4.20	5.65	1.32	2.91	5.60	7.65	2.20	4.20

digitalis, xanthines, and mercurials showed clear-cut differences, indicating different modes of action. The creatinine clearances during digitalis and xanthine diuresis pointed to increased glomerular filtration with very little decrease in tubular reabsorption; while after injections of mercurials, diuresis was accomplished primarily by decreasing the tubular reabsorption of fluid, with no apparent increase in glomerular filtration. The chlorides seemed to remain at about the normal level in the blood, but the urinary output was greatly increased. Blood CO_2-combining power dropped as base was lost through the kidney. The urea levels rose conspicuously and dropped off promptly to just above normal. The urinary excretion of urea rose at the height of diuresis and fell below the normal level after ten hours (Herrmann, Schwab, and Bondurant, 1932).

The data that were obtained seemed to point to a primary effect of xanthines and mercurial diuretic drugs directly upon the kidney units and probably upon the local circulation. The digitalis effects were mediated through improvement in the general circulation. Aminophyllin seemed to accomplish diuresis primarily by increased glomerular filtration, possibly mediated by afferent arteriolar dilatation. Salyrgan on the other hand seemed to produce diuresis primarily by decreasing tubular reabsorption probably by efferent arteriolar vasoconstriction but more likely by toxic decrease in permeability of the tubular epithelial cells in the kidney.

The Rehberg creatinine clearance method for calculating glomerular filtration and tubular reabsorption was admittedly open to some question in humans but was defended by some physiologists (White and Monaghan, 1933). Shannon, Jolliffe, and Smith (1932) investigated the use of various possible nonthreshold substances, such as inert sugars, for the determination of glomerular filtration and recommended d-xylose and inulin clearances as the best indices of this function. We repeated our studies with concomitant observation of the clearances, of creatinine, inulin, d-xylose, urea, and chloride during diuresis in patients with edema of congestive heart failure. Our results confirmed our previous contentions. We, therefore, reiterated our conception of the mechanism of diuresis in

patients with congestive heart failure (Herrmann, Schwab, Alvarez, and Cate, 1933) (Fig. 1).

This conception of two different areas of action for the two different types of diuretic drugs led to the idea that the drugs clinically might complement each other. Various combinations of oral and intravenous diuretic drugs were tried out. Extensive clinical studies proved the great advantage of alternating the vegetable and metallic diuretics in the treatment of edema of congestive heart failure (Herrmann, Schwab, Bondurant, and Stone, 1932; Herrmann, Schwab, Marr, and Stone, 1933).

The practical value of the complementary action of the anti-edemic drugs was further reported upon by Herrmann, Schwab, and Stone (1933), Herrmann and Decherd (1937), and Herrmann (1939, 1940).

Sodium and Chloride Studies

Sodium and chloride have long been recognized as significant urinary excretory products. However, there has been much argument as to whether the chloride ion or the sodium ion is the more important in body water balance. The significance of the ratio of sodium to chloride in tissue fluid status and distribution has been recognized. Volhard has held indisputable the hydropigenous property of the chloride ion particularly in its relation to water metabolism in the tissues,

TABLE II

Summary of Clinical Diuretic Effects

Drug	No. Cases	Average Increase in Output of Urine			
		First Day	Second Day	Third Day	Total for 3 Days
Mercupurin	611	476.8	151.6	79	707.4
Salyrgan	125	341	120	62	526
Salyrgan and Theophyllin	75	335	106.6	42.2	483.8
Esidrone	41	355	36	21	412
Mercupurin and Aminophyllin	10	415.1	154.5	32.8	562.4
Aminophyllin	29	263.3	118.8	53.6	435.7
Theophyllin and Sodium Acetate	6	29.5	59.1	26	114.6
Decholin	22	83.1	34	10.6	127.7
Decholin and Mercupurin	7	770	101	7	878
S. T. O. 3813	5	240	80	20	340
Mercurin Suppositories	70	204	66	25	355
Decholin, Salyrgan and Theophyllin	1	900	160	300	1360

which he so aptly designated *Vorniere*. His contention was supported by his student, Weingarten (1932).

The sodium ion, however, was incriminated in tissue fluid retention by Presser and Stahl (1933). It was found that the edema producing effect of sodium could be set aside by a balanced combination of potassium, calcium, and magnesium ions. This observation was used as the basis for the development of a substitute for table salt for edematous patients. A good correlation between sodium excretion and urinary output has been established by Bruman and Jenny (1934). Greater retention of sodium than of chloride in the edema fluid and in transudates in the pleural and peritoneal cavities in congestive heart failure was demonstarted by Siedek and Zuckerkandl (1935). Such was evident in the edematous cardiac patient in the diuresate of whom there appeared conspicuous excesses of sodium over chlorine.

The study of the chlorides and water excretion after the intravenous injection of mercurials into the patients with diabetes insipidus convinced Bua (1935) that the presence of chlorine was the *sine qua non* requirement for diuretic action of organic mercury salts. Simmert (1935) observing the effects of diuresis produced by organic mercurials, in patients with and without edema, noted increases in the output of sodium chloride and concomitant rise in blood plasma sodium chloride, determined as chloride, along with decreases in the hemoglobin and erythrocytes. Hitzenberger and Engleman (1935) observed during organic mercurial diuresis augmentation of excretion of many inorganic and organic substances particularly chloride.

In our laboratory (1937) we were much interested in the attempt to elicit the initiating factors, those at work during the first five or six hours of diuresis. Urinary Na/Cl ratios were determined before, hourly during, and in a follow-up for several days after each of 25 diuretic experiments with aminophyllin, mercupurin, and salyrgan. The initial drop in the plasma sodium chloride was not satisfactorily demonstrated. In general the blood sodium remained unchanged, but the urinary sodium increased conspicuously. The increase, however, was not in tremendous proportions of the chloride

excretion, so that the Na/Cl ratios were as a rule decreased. Occasionally, however, the reverse was noted, and Na/Cl ratio increased. In these studies neither the mercurials nor the xanthines show consistent or characteristic effects of the Na/Cl ratio.

The excess sodium and chloride mobilized from the tissues during diuresis was calculated, and a fair approximation established by subtracting volumes of urine with its contained sodium chloride during the control hours and the corresponding figures obtained after the injection of the diuretic. Assuming the edema fluid to be an ultra-filtrate of blood plasma, the excess amount of sodium and chloride that should be excreted with the excess excretion of urine of the same composition as tissue fluids could be calculated. The values for sodium determined on this basis showed a good agreement with those actually found, while the actual excretion of chloride was in most instances greatly in excess of the calculated values. Sodium excretion during diuresis then agreed well with the amount of sodium present in an equivalent amount of edema fluid while chloride excretion failed to show this agreement.

Blood Plasma Volume Studies

It seemed quite evident from our studies of water shifts, blood protein levels, and sodium and chloride excretion during diuresis, that there must be some accompanying changes in the blood plasma volume. We were, therefore, naturally led to make observations along these lines. Meyer (1933) postulated as a basis for diuresis a physicochemical water-freeing effect upon the tissue colloids, and the resulting hydemic plethora in turn was said to dilate the renal vascular bed and account for the actual outpouring of the urine. With Calvin (1940) we attempted to establish plasma volume shifts. Studies were carried out on specific gravity, hematocrit volumes, hemoglobin and albumin, and total protein level shifts, as well as the volume changes indicated by the dilution of the Evans Blue dye, T–1824, according to the method of Gregerson, Gibson, and Stead as modified by Gibson and Evelyn (1938).

The results of these studies, which will be given in detail in another paper, revealed trends of plasma volume shifts that seem to be definite. In the experiments following injection of the mercurial, salyrgan, intravenously there was practically always a concentration of plasma volume that seemed to parallel diuresis. Careful studies using aminophyllin (theophyllin ethylene-diamine) intravenously revealed a strikingly different blood volume plasma response. In only two experiments was there demonstrable a slight momentary decrease, and a sharp conspicuous early increase was regularly present. During the time of the profuse diuresis there was a characteristic tendency for the plasma volume to return towards the initial level. If the aminophyllin diuresis continued, there was a shift of the plasma volume apparently dependent in part at least upon the balance established between the tissue fluid mobilization and the renal output. The usual result was a blood plasma volume lower than that initially observed. The blood plasma volume is usually abnormally high in the presence of congestive heart failure.

Blood Serum Protein Studies

In our earlier studies we noticed a definite association of low plasma proteins in patients with congestive heart failure and edema. We, furthermore, found that blood plasma proteins did not promptly rise as the edema was dissipated by a diuretic and as the patient spectacularly improved otherwise. Apparently slow elaboration of blood plasma proteins was associated with the chemical changes in the liver incident to chronic passive congestion. Shally (1934), after some confirmatory physiochemical studies, accepted the conclusion of Nonnenbruch (1933) and others that changes in the colloid osmotic pressure in the blood plasma did not explain the movements of water in diuresis.

However, Shally (1934) reported an absolute and relative increase in the albumin fraction, lowered and unchanged globulin values, and a drop in the fibrinogen level at the onset of diuresis in all cases. He tried the effect of salyrgan on the blood serum in vitro and concluded that in the concentration

that it appeared in the blood after injection, salyrgan produced no change in the fractionation of serum proteins. He admitted that at very high concentration of organic mercurial in the blood serum, there was some flocculation. Bieter and Wright (1935), on the other hand, demonstrated with the ultramicroscope definite physical changes in the blood serum of patients who had received therapeutic doses of salyrgan.

In recent studies in our department of biochemistry and in our laboratory for clinical research, Calvin, Decherd, and Herrmann (1940) have found in patients with congestive heart failure increases in total circulating plasma protein during aminophyllin diuresis, slight rises following the exhibition in digoxin. After salyrgan intraveneously, with the drop of plasma volume there is a rise in the percentage of total protein, predominantly albumin, and thus a rise in the albumin-globulin ratio. The findings suggest probable extrarenal fluid mobilization during aminophyllin effects; during salyrgan diuresis the plasma protein is concentrated, and after diuresis percentage increase persists, but the total is lowered. Plasma protein concentrations (Calvin, Decherd, Herrmann, 1940) during diuresis were found to shift rather rapidly into, and later out of, the circulation, suggesting that there must be some degree of back flow of proteins as well as electrolytes and water through the capillary walls in some parts of the capillary bed, possibly in the liver.

Summary

The significant anatomical facts concerning the functioning unit of the renal parenchyma and the chief physiological relationships and factors in the maintenance of water balance have been reviewed. The role of the kidney in the physiology of secretion of the urine and the relationship of the chemical constituents of the circulating blood plasma and the urine have been stressed. The various theories of kidney function are analyzed. The circulatory contributions to the nervous and hormone factors in fluid equilibrium in the interstitial tissues have been touched upon.

In our early studies of the total blood plasma protein, serum albumin and globulin were found at depressed levels in patients with congestive heart failure. Evaluations of shifts of water and salt were undertaken in patients with edema of congestive heart failure into whom powerful diuretics had been injected. Such experiments were planned with the hope of determining changes in the constituents of the blood which are so very little, under the normal conditions of urine secretion, that present micromethods are inadequate.

The studies of the effects of diuretics seemed to point to a direct effect of drugs upon the kidneys, probably chiefly on the circulation into the glomeruli and about the convoluted tubules. Creatinine clearance studies during diuresis produced by various types of diuretic drugs strongly suggested that each type acted in a specific way and probably on a different part of the renal unit. Xanthines seemed to produce diuresis primarily by increasing the blood flow in the glomerular tuft with resulting increase in glomerular filtration. Mercurials, on the other hand, during maximum diuresis, gave evidence of primary decrease in tubular reabsorption. This temporary decrease in ability of the tubular epithelium to absorb water and salts was considered to be a direct effect of the mercury on tubular cells. Digitalis seemed to act by improving the general circulation, apparently thereby increasing the glomerular blood flow.

D-xylose, inulin, and urea clearance studies seemed to have confirmed findings of the creatinine clearance studies.

On the basis of the different points of action of the xanthines and the mercurials it seemed logical to try combinations of drugs for more effective diuresis.

The clinical experiment seemed to corroborate the theoretical consideration, for the combinations were definitely more effective in the production of diuresis than either one of the preparations given alone, intravenously or by other routes.

The sodium and chloride ion were found to play important roles. Sodium increase during diuresis agreed well with the amount calculated to be present in an equal amount of edema

fluid. Chloride increase on the other hand failed to show this agreement.

Total circulating blood protein, particularly albumin, showed moderate increases during the stage of increase of blood volume in diuresis. A significant loss of proteins, chiefly albumin, from the circulating plasma was recorded as plasma volume dropped off.

The tremendous augmentation of the blood plasma volumes following intravenous aminophyllin must be considered a factor in the production of diuresis, probably accounting for the beneficial effects of this therapy in peripheral vascular disease. Mercurial diuretics alone, on the other hand, uniformly showed a shrinkage in blood plasma volume during maximum diuresis in the presence of good renal function.

Our application of newer and more accurate methods to the study of relatively old fundamental problems of urine secretion, left most questions of the mechanisms involved still unanswered. Our extensive diuretic studies have yielded some interesting and practical data, which have helped us to make more nearly rational our treatment of edema. Our concept of the pathological physiology of water and electrolyte metabolism in patients with congestive heart failure has been clarified.

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BLOOD PLASMA VOLUME AND SERUM PROTEIN STUDIES DURING DIURESIS*

GEORGE M. DECHERD, D. BAILEY CALVIN, AND GEORGE R. HERRMANN

For over a decade this laboratory has carried forward clinical experimentation directed toward a better understanding of the initiation and mechanism of diuresis, particularly as it applies to the acceleration of fluid excretion in patients with congestive heart failure. This endeavor has led us into many aspects of the problem, perhaps the most fruitful of which has been the demonstration that mercurial and xanthine diuretic agents act at different points in the renal excretory unit, thus affording a rational basis for their combined utilization, the value of which has been proven by extensive clinical experience (Herrmann and Decherd, 1937). Further studies along this line are still in progress.

We have long sought to find the earliest change, slight as it might be, in the complex homeostatic equilibrium, which might initiate the increased urinary flow. However, we have been unable to detect any change in either colloid or crystalloid blood constituent to which we could point as the factor, which, by upsetting the existing balance, set off the diuresis. From a study of the renal processes as manifested by clearances and from a study of the individual components of the blood plasma, we have, therefore, turned to a study of the total circulating blood plasma. We hoped that the measurement of this factor before, during, and after diuresis, might give further clues to the intricate mechanisms involved in the processes of fluid mobilization and excretion. It is our purpose here to describe in detail our observations on changes in the blood and plasma volume in patients suffering from congestive heart failure during the course of diuresis induced by various agents. A preliminary report of these studies has been made (Herrmann, Decherd, and Calvin, 1941). The

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multiplicity of factors which may conceivably influence the rate of urinary flow has been summarized elsewhere (Herrmann, 1940).

The effect of diuretics and of diuresis on blood volume has been previously studied, but the results have not been conclusive. The earliest such study was that of Brown and Rowntree (1925), but the fact that they employed a red dye is enough to explain their failure to demonstrate constant changes in plasma volume incident to diuresis. Feher (1929) found a marked increase in circulating blood volume at the height of diuresis induced by salyrgan. In one case in which the drug failed to produce a diuretic response, there was no change in blood volume. These results were confirmed by Saxl and Erlsbacher (1930), who noted that although salyrgan produced a rise in blood volume, the additional administration of ammonium chloride caused a decrease. Plesch (1933) has also reported an increase in blood volume following the action of a mercurial diuretic, ascribing this to the increased capacity of the blood vessels after the dissipation of edema. Hitzenberger and Tuchfeld (1929) found no constant change in blood volume after the treatment of cardiac failure with digitalis and salyrgan; and Borruso and Ceccarelli (1924) observed first a decrease, followed by an increase, when salyrgan was employed.

Goldhammer, Leiner, and Scherf (1935) employed both the trypan-red and carbon monoxide methods of estimation of blood volume. They concluded that mercurial diuresis was accompanied by a decrease in the volume of the circulating blood. They followed the idea advanced by Plesch and believed that removal of excess fluid from the tissues enlarged the peripheral blood depots to which blood might be transferred from the engorged liver and venous system. More recently this problem has been studied by Swigert and Fitz (1940). Although their method, employing Evans blue, is acceptable, their results are highly variable. We have suggested (1941) that this may be due to the fact that their second blood volume estimation was made at a time when the blood volume was not stationary but was in the process of changing.

Our own studies of this question have been made with Evans blue dye, T–1824, using the method of Gregerson, Gibson, and Stead (1935), as described by Gibson and Evelyn (1938) for the photoelectric microcolorimeter and modified so that serial values for blood volume could be calculated. The night before the experiment the dye was injected in the dosage of 0.3 mg. per kilo. By the following morning, the disappearance curve of the dye from the blood stream approximated a straight line. Three determinations of the dye concentration served to establish the slope of the disappearance curve. Variations from the curve thus established were taken to represent changes in dye concentration due to shifts in plasma volume associated with the diuretic response to the drug employed.

After determination of the control slope of the dye disappearance curve for the individual patient, the drug to be studied was injected. Blood samples were then collected at intervals for periods up to 12 hours. During this time the rate of urine flow was also recorded. We suspect that our absolute values for plasma volume after eight to twelve hours are probably somewhat low, since the disappearance slope probably deviates slightly from a straight line. Correction of these values would be desirable before termination of the experiment, and this could be done by reinjection of dye if the plasma volume were nearly static. However, such a static level of blood volume is not attained within these periods; Evans and Gibson have pointed out (1937) that under such circumstances reliable determinations cannot be attained. This attempt to estimate changing plasma volume by such reinjection has been made by Swigert and Fitz with unsatisfactory results.

Meanwhile we studied serum proteins during diuresis in an effort to explain the conflicting data previously available. Herrmann (1932) noted that serum protein was low in patients with congestive heart failure; the drop was in the albumen fraction, with a slight rise in the level of the globulin. The lowered values tended to rise after the edema disappeared. Shortly afterwards, Shally (1934) investigated the alterations of blood protein during salyrgan diuresis. He

undertook first to evaluate the possibility that the mercurial drug induced a physico-chemical change in the protein. Mixture of salyrgan with blood plasma in vitro was found to alter the dispersion of the protein aggregates, changing the smaller colloidal particles to larger ones. Such a change in dispersion might readily affect fractionation, so that the coarsely dispersed albumen would precipitate as globulin. However, such behavior could not be shown to occur in salyrgan-plasma mixtures that would be possible in a living organism. In this connection it should be noted that Bieter and Wright (1935) demonstrated ultramicroscopic changes in the serum proteins of patients who had received therapeutic doses of salyrgan.

In his clinical studies, Shally found that after diuresis there was an absolute and relative increase in serum albumen with little or no change in the globulin fraction. He considered that this was due to an actual increase in the total circulating blood albumen, which was a result, not a cause, of diuresis. He concluded that the mercurial diuretic affected the capillary walls so that fluid entering the blood stream brought albumen with it during the water mobilization associated with diuresis.

Experimental Data

The substances employed as diuretic agents were aminophyllin (12 experiments), salyrgan (11 experiments), mercupurin (4 experiments), and digoxin (5 experiments).

Plasma Volume Changes

Aminophyllin (0.5 gm.) was injected intravenously, diluted in 20 cc. of physiological saline. Four patients without heart disease and eight with congestive heart failure were studied. Representative curves are shown in Fig. 1 for one control study and four studies in edematous patients.

The typical response to the drug appeared to be a slight initial drop within the first 30 minutes, followed by a significant rise in the plasma volume. This rise was typically interrupted by a transient drop, which seemed to be correlated

with the loss of fluid by renal excretion. Usually the plasma volume continued to rise, in spite of the fact that the rate of urine flow soon dropped off; this rise was then followed by a large drop, which also bore no relation to urine volumes. We regard the plasma volume curve shown by patient A. O., Fig. 1, as fairly typical except for the absence of the initial

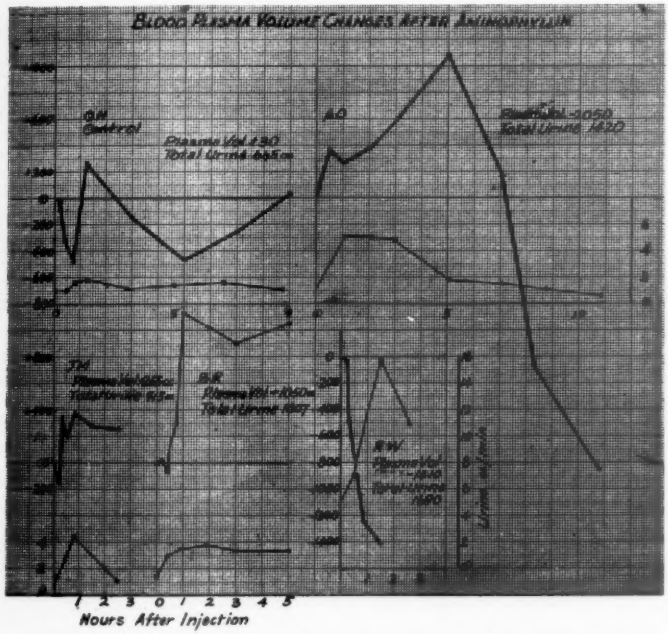

Fig. 1. Blood plasma volume changes after aminophyllin. In this and successive figures the heavy line shows increase (+) or decrease (—) in plasma volume from control levels. The rate of urinary flow is recorded in cc. per minute by the light line. The abscissae are hours after injection of drug.

slight drop in volume. This "type-curve" may of course be modified by several factors.

The availability of excess fluid in the tissue interstices, the failure of significant diuretic response, or its rate when present, would reasonably be expected to affect the volume of circulating plasma. Thus, in patient O. H., who had no

edema, the initial drop in plasma volume was followed by only a slight rise, presumably because there was not much fluid in the tissues available for mobilization. In patient R. W., the extremely rapid loss of fluid through renal excretion virtually drained the plasma water off at a rate which much exceeded the rate of flow from the tissue spaces into the circulating plasma, resulting in a net loss of plasma volume of marked degree.

At the peak of diuresis there was characteristically a drop in the volume of the plasma. Other factors, which appear to be largely extrarenal and probably are operative in the tissues, play the chief role in determining the reaction of the plasma volume to aminophyllin. What these factors are and the exact site of their effect remain an open question. The many possible modes of action have been reviewed elsewhere.

Salyrgan (2 cc., injected intravenously) was studied in three control patients and in eight patients with cardiac disease. Here again, the data indicate both renal and extra-renal actions of the drug. An initial transient drop was seen on several occasions, e.g., in patients Sch. and L. S., Fig. 2. This was lacking in other tests, perhaps because of failure to take samples early enough. The transient drop was typically followed by a rise in the volume of circulating plasma. In one patient, Sch., there ensued a large drop, which may be correlated with the concomitant diuresis. The corresponding drop noted in other patients cannot so readily be associated with diuresis; however, where the diuresis was extremely rapid, as in patients L. H. and Vu., the observed drop in plasma volume was clearly due to fluid loss through the kidneys.

In one patient of the series, although edema was present, injection of salyrgan failed to produce a diuresis. Measurements of the plasma volume showed only a moderate rise. The data indicate that when salyrgan was used, the blood volume was influenced by two factors: the loss of fluid by renal excretion; and the mobilization of fluids from the tissues into the blood stream.

Mercupurin (2 cc., injected intravenously) is a mercurial-xanthine combination which has proven to be a most efficacious diuretic. Its effect on the kidney, as measured by cre-

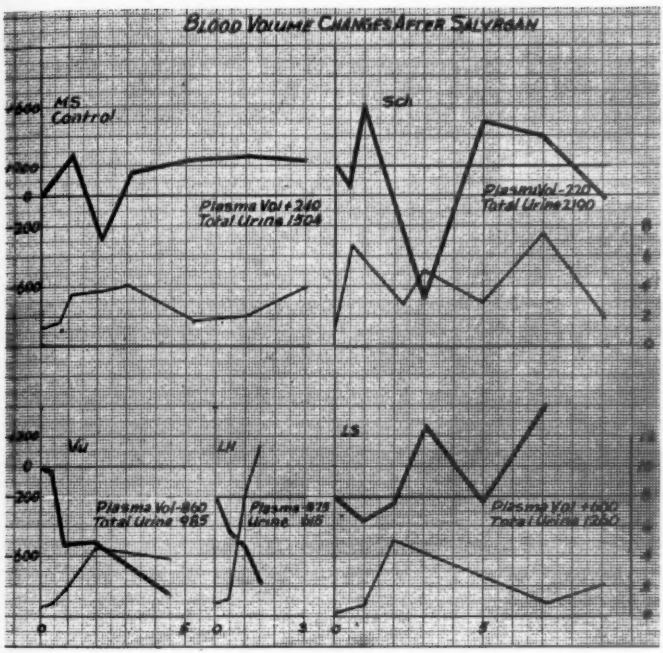

Fig. 2. Blood volume changes after salyrgan.

atinine clearance studies, is identical with that of its mercurial component (Herrmann and Decherd, 1937). The effect of the combination drug on the plasma volume, however, seems to be predominantly that of its xanthine component.

Curves shown in Fig. 3 show the typical features in the four cases studied, an initial decrease of variable degree followed by a rising plasma volume interrupted by a drop associated with diuresis. The later portion of the curve is variable, probably depending on the loss of fluid by diuresis, the excess of fluid in the tissues, and probably other unrecognized factors.

Digoxin in appropriate dosage, varying from 1 to 2 mg., was injected intravenously in five patients; the data from four cases are shown in Fig. 4. In patient A. the gradual action

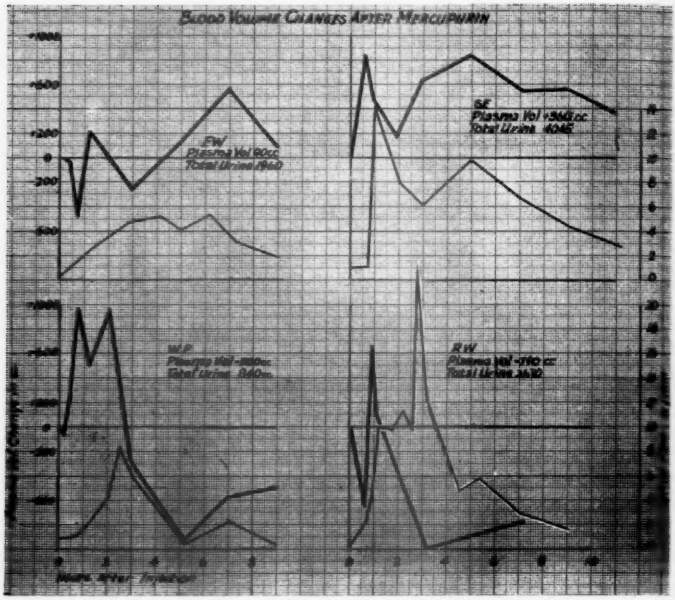

Fig. 3. Blood volume changes after mercupurin.

of the glucoside is evidenced by the gradual increase in the rate of urinary flow. The first effect on the blood volume in this patient, as well as in patients H. E. and H. B., was a slight increase. In A. and H. B. the persistent acceleration of urine flow caused an ultimate decrease in plasma volume, though during the first six or eight hours after injection the volume was maintained almost stationary, presumably by the entrance of fluid from the tissue spaces into the blood stream at a rate which kept pace with renal fluid excretion.

In patient H. E. diuresis was moderate and transient, and the mobilization of tissue fluid exceeded fluid excretion, so that the drop in plasma volume during diuresis was succeeded by a rising plasma volume. Patient S. C. had been in bed for five days, and probably was close to a spontaneous diuresis. Injection of digoxin produced a diuresis of 4,270 cc. in 12

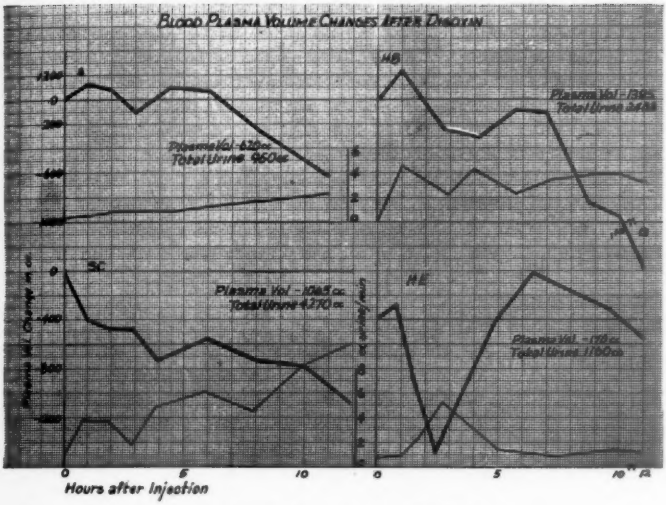

Fig. 4. Blood plasma volume changes after digoxin.

hours. The loss of fluid was so rapid that no plasma volume rise was detected, a progressive drop being recorded, which amounted to 1,065 cc. at the end of 12 hours. The difference, 3,200 cc., was undoubtedly withdrawn from the tissues.

Blood Plasma Proteins

The changes in blood protein may be illustrated in three typical cases (Table I). In patient M. V., injection of salyrgan caused a marked drop in plasma volume during a five-hour period of observation. The total protein content of the serum rose in a reciprocal fashion; the increase was due chiefly to an increase in albumen, so that there was a rise in the albumen/globulin ratio. When the total circulating blood protein is studied, however, it may be seen that there has actually been a large loss of protein from the blood stream. The changes of th A/G ratio are not due simply to concentration or dilution of the blood, since the ratios are not affected by serum dilution in vitro.

Plasma Protein Shifts During Diuresis

TABLE I.

Drug	Hr after injection	Plasma volume, cc	Total protein		Albumin		A/G ratio
			g%	g total circulating	g%	g total circulating	
Salyrgan	0	5140	5.74	295	3.13	162	1.20
MV	½	5110	5.91	302	3.26	166	1.23
	1	4620	6.02	279	3.45	159	1.34
	2	4620	6.11	283	3.46	161	1.31
	5	3280	6.20	205	3.63	119	1.42
Aminophyllin	0	6120	6.10	373	3.38	207	1.24
AO	½	6488	6.05	393	3.34	223	1.22
	1	6383	5.94	380	3.32	212	1.27
	2	6500	hemolysis				
	3	6697	6.01	402	3.39	227	1.30
	5	7202	5.87	423	3.43	242	1.41
	7	6278	6.25	393	3.63	228	1.39
	9	4913	6.39	314	3.62	178	1.40
	11	4072	6.44	263	3.81	156	1.45
Digoxin	0	3890	5.76	224	3.24	126	1.29
HB	1	4130	5.62	232	3.24	134	1.36
	2	3640	6.00	218	3.50	127	1.40
	3	3682	5.87	216	3.36	124	1.34
	5	3800	5.63	214	3.22	122	1.34
	7	3790	hemolysis				
	9	3060	5.94	182	3.63	111	1.57
	11	2505	6.06	152	3.65	92	1.51

Augmentation of the blood volume by injection of aminophyllin in patient A. O. caused a drop in plasma protein percentage but an actual increase, of considerable magnitude, in the amount of protein present in the blood stream. This increased amount of protein was chiefly albumen. In patient H. B. injection of digoxin caused a drop in plasma volume with changes in the serum protein similar to those seen after the administration of salyrgan.

It may be seen that the changes in blood protein during or after diuresis depend in large part on the status of the blood volume at the time of sampling. These findings permit of rational interpretation of the divergent results obtained by previous investigators.

COMMENT

Diuresis after the administration of digoxin is probably due to improvement of myocardial function by the drug, restoration of cardiac compensation, reduction of venous pressure,

and secondary increase in the rate of urinary flow. There is no uncertainty about the site of the action that leads to diuresis—it is on the heart muscle. The slight increase in blood volume is due to reduction of venous pressure, which allows more fluid to enter the blood stream from the tissue spaces than filters in the opposite direction. As the diuresis develops, there is some drop in plasma volume; fluid mobilization from the tissues almost keeps pace with fluid excretion, eventually lagging behind as the diuresis continues, and as the amount of edema fluid diminishes. The end result is a lowered plasma volume. The initial rise in plasma volume precedes the diuresis, but probably *per se* is not responsible for it. Injection of 100–200 cc. of saline or plasma does not lead to diuresis in a patient with heart failure. Hence, the preliminary rise in plasma volume after the injection of digoxin cannot be the essential cause of diuresis. Bettered oxygen supply to capillary walls in tissues and kidney may play a role, but many other factors remain to be discerned and evaluated.

The mercurial and xanthine diuretics act on both the renal excretory units and on the tissues or blood stream. We have previously studied their mode of action on the kidney; this is reflected in the blood by a decrease in plasma volume as fluid is excreted by the kidney. A rise in plasma volume, seen to a limited degree after injection of salyrgan and strikingly after the injection of aminophyllin and mercupurin, cannot be explained on the basis of any renal effect. The rise must be due to extra-renal compensatory factors; our data do not allow any exact localization, though various possible sites have been proposed—on tissue colloids or crystalloids, on capillary walls, on components of tissue fluid, or on constituents of the tissue cells themselves. The extra-renal effect predominates after the injection of a xanthine, resulting in a plasma volume rise. This rise is interrupted by a transitory drop which may be clearly associated with the accompanying diuresis. The increase in plasma volume may explain the occasional untoward effects which follow the intravenous administration of aminophyllin, such as pulmonary edema.

The fact that mercupurin resembles salyrgan rather than aminophyllin in its effect on renal clearances has led us to

suggest that the drug be fortified by the employment of additional xanthine. Therefore we are especially impressed by the fact that when judged by changes in blood volume, the mercupurin effect is predominantly that of the xanthine, aminophyllin. The variation in the type of action at renal and extra-renal loci may further clarify the clinical efficacy of mercupurin.

The initial drop in plasma volume, seen in many instances during the first 30–60 minutes after both types of diuretic drugs, may be a part of the initial changes that lead to diuresis. Further study will be necessary, however, before we are able to detect satisfactorily the trigger mechanism which sets off the increased flow of urine.

The changes in the blood protein concentration observed during and after diuresis are consistent with previous experience with protein shifts with changing blood volume (Calvin, 1933, 1941). Marked hydremia lowered the percentage concentration of plasma protein but increased the total amount in the circulation, particularly the albumen fraction. Conversely, shock due to hemorrhage or asphyxia decreased the total blood volume and the total circulating blood protein, though the concentration of the latter within the serum was increased. It is in the albumen fraction that these changes take place. The blood volume shifts incident to diuresis led to similar changes in blood protein. Thus, decreased blood volume was associated with increased protein percentages, though the total circulating protein was diminished. Increased blood volume affected the blood protein in the opposite direction. This lability of blood protein seemed to involve chiefly a shift in the albumen fraction. These observations suggest that blood protein, particularly albumen, may be rapidly mobilized from a tissue depot or as rapidly stored there, when fluid shifts require hemostatic responses on the part of colloidal components for the maintenance of osmotic pressure equilibrium.

Summary

Changes in the plasma volume have been studied during diuresis induced by salyrgan, aminophyllin, mercupurin, and digoxin. These changes do not appear to cause the diuresis.

On the contrary, they simply reflect the balance of fluid mobilization from the tissues into the circulating plasma, and fluid loss from plasma into the tissue spaces or by renal excretion.

Changes in blood plasma protein concentration depend largely on shifts in plasma volume and take place in the direction required for approximate maintenance of colloid osmotic pressure.

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THE COMPARATIVE EFFECTS OF THE SUBCUTANEOUS INJECTION OF QUITENINE ON THE KIDNEYS OF GLOMERULAR AND AGLOMERULAR FISH*

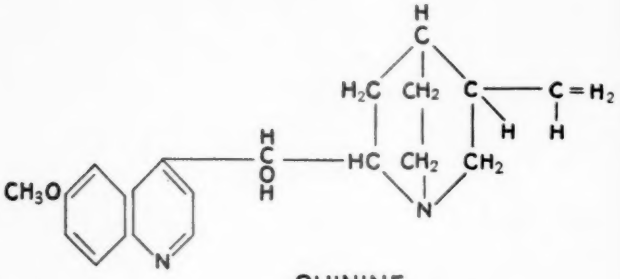
CHARLES H. TAFT AND JESSE A. PLACE

Marshall (1930) showed that while in general most substances were handled in the same manner by the glomerular and aglomerular kidney, certain substances were not excreted by the aglomerular kidney. He also (1934) called attention to the value of studying glomerular and aglomerular kidneys in relation to theories of renal function. We thought it might be of interest to determine if there were any difference in the reaction between the glomerular and aglomerular kidneys to a substance such as quitenine, which is known to be destructive to tubular epithelium but apparently has little action on the capsule.

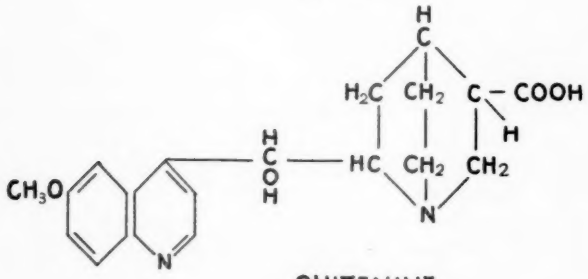
When quinine is treated with potassium permanganate, the vinyl group is oxidized to a carboxyl group yielding quitenine ($C_{19}H_{22}O_4N_2.4H_2O$) Von Oettingen, 1933) Figure 1. This is probably the same as the dihydroxyquinine of Kerner (1870).

While this substance does not possess great pharmacological activity, considerable work has been done with it. Herschfelder, Jensen, and Swanson (1923) found it to be less toxic to rats than quinine and to have some antiseptic properties. Dixon and Premankur (1927) confirmed this and announced that it possessed local anesthetic properties probably due to its effect on the nerve endings. Giemsa, Weise, and Tropp (1926) state that it is ineffective against avian malaria. Dauber (1920) reported on the effect on the rabbit kidney. When the urine was kept acid by a diet of oats, there were casts, albumen, and blood in the urine, and the epithelium of the tubules was damaged. Lipkin (1919) inoculated fresh sheep and guinea pig liver with quinine for 18 hours and found that a part of the quinine had been converted to quitenine.

*From the Departments of Pharmacology and Anatomy, The University of Texas Medical School, Galveston, Texas, and the Laboratories of the Marine Biological Laboratory, Woods Hole, Massachusetts. Submitted for publication October 30, 1943.



QUININE



QUITENINE

FIGURE 1

Dawson, Vos and Little (personal communication) noted that deaths from quitenine occurred later than with other cinchona bases (5–7 days as compared with 1–2 days). Rabbits given 2 mgm./mol./kgm. subcutaneously showed oliguria or anuria for 24–48 hours. Urine later contained red blood cells and albumen. These rabbits were sacrificed on the 15th day, and the kidney volumes compared: mean kidney volume of quitenine rabbits 13.2 ± 1.1 cc.; mean kidney volume of control rabbits 8.0 ± 0.5 cc.; the difference is statistically significant. The kidneys of the quitenine rabbits were a peculiar café-au-lait color. Kerner (1870), Halberkann (1919), and Nierenstein (1919) claim to have isolated quitenine from the urine of people taking quinine. Of the various species of vertebrates injected subcutaneously with quitenine all except the frog show large sloughs at the site of the injection (Taft, 1940).

Quitenine base is quite insoluble, so it was injected as the dihydrochloride. Quitenine 1.035 gms. plus 2.5 cc. 2 N hydrochloric acid was made up to 10 cc. with distilled water, giving a ¼ molar solution or roughly a 10% weight-volume solution. When this is injected in the dose of 8 cc./kgm., one has injected 2 mgm./mol./kgm.

Marshall (1929) has shown that the kidney of the common toadfish *(Opsanus tau)* is aglomerular. It was found that this fish stood captivity well and would survive several weeks in the tanks so it was used as an example of the aglomerular fish.

Marshall and Smith (1930) have shown that tautog *(Tautoga onitis)* has a glomerular kidney. This fish also survived several weeks in the tanks and was used as an example of the glomerular fish.

The fish used were kept in the portable glass tanks at the Marine Biological Laboratory and were supplied with running sea water at all times. They were not given food during the experiment. They were kept in the tanks a few days before being used.

Procedure

The fish were removed from the tank wrapped in a tared towel, weighed rapidly, and then injected subcutaneously about 2 cm. laterally to the dorsal fin and returned to the tank. The doses used for both species were 4, 8, and 16 cc./kgm. of the ¼ molar solution of quitenine dihydrochloride. The fish were out of their native element probably about a minute and a half on the average and did not show any evidence of damage from having been out of the water for this length of time.

After varying intervals of time, the fish were removed from the tank, killed with a blow on the head, and rapidly autopsied. If there was any urine in the bladder, it was examined. The kidneys and livers were removed and immedidiately placed in Bouin picro-formal solution for a week and then transferred to 70% ethyl alcohol in which they were kept. Sections were cut 6 μ thick and stained with hematoxylin and eosin or iron hematoxylin.

The fish used were of either sex and varied in weight from: for the toadfish 100 to 620 grams and for the tautog from 146 to 587 grams.

A total of 52 toadfishes were injected with different doses of quitenine. These all showed sloughs at the site of injection in a day or so. The 38 tautogs injected with different doses also showed sloughs. These sloughs varied in size from about that of a quarter to more than 2 inches in diameter. They did not apparently bother the fishes very much. They were able to swim about normally. Due to the long lasting local anesthetic action of quitenine they were probably painless. There was no visible sign of pus or fungus infection in the sloughs. It is very probable that the water and salt metabolism was upset by this injury to the skin. It seems very doubtful whether this interfered in any way with the results of these experiments as there were sloughs of equal size in both types of fish and any error introduced by this source should be equal in each case.

Gross Observations

In the toadfish on examining the viscera, there were no abnormal conditions in the circulatory system or intestinal tract. In a few cases with the dose of 16 cc. per kilogram the liver was very mushy. The gall bladder in the normal toadfish appeared a blueish purple. In the injected fish the color varied: bluish purple, yellow, green, pale pink, orange, various shades of green, and brown. There was no apparent correlation between the color of the gall bladder, the size of the dose or the length of time after injection that the fish was killed.

The kidney of the toadfish is normally a rather firm dark red organ lying in the dorsal part of the body cavity close to the vertebral column and showed marked changes after the injection of quitenine. The kidneys became swollen, and soft, and frequently took on a browish color. In a few cases the kidneys were mottled, red and white. Here there seems to be a direct correlation between the severity of the change and the size of the dose and the length of time the animal

was allowed to survive, those living the longest time (7 days) showing the greatest change.

In all cases where the urine was examined, there were red blood cells and epithelial cells present, regardless of the size of the dose or the length of the survival. In addition there were crystals in the urine and in many cases almost solid crystalline masses in the ureters. In most cases these crystals were the ordinary crystalline components of urine, but occasionally there were crystals closely resembling quitenine. There were not enough of these to do any crystallography tests on. The urine responded positively to the qualitative tests for quitenine. The pH of the urine ranged from 6.5 to 7.5. In only one case was it above 7.

In the tautog on gross examination, the picture was essentially the same as with the toadfish. There was no damage to the circulatory system or digestive tract. In one or two cases the liver was abnormally soft. In all cases the gall bladder was a greenish blue, as it was in the uninjected fish. The kidney in many fish was pink rather than the normal dark red, occasionally was mottled with white spots, and at times was quite soft. The urine showed red blood cells, epithelial cells, and many small crystals. There were no crystalline masses in the ureters. The urine gave a positive qualitative test for quitenine. The pH was about 7.0.

We were unable to find any description of the type of epithelium occurring in the tubules of the tautog kidney. So, before starting a study of the microscopic pathology a careful study was made of the normal tautog kidney. This was done by studying serial sections of normal tautog kidneys, and the following divisions of the tubule weres recognized: neck segment, proximal segment and initial collecting tubule. The nephron of the tautog seems to be essentially the same as that of the sculpin described by Grafflin (1937).

Microscopic study of sections cut from the kidneys of tautogs that had received different doses of quitenine and were allowed to survive for varying lengths of time showed little or no capsular damage (Plate 1, Figs. 1 and 5), and pathological changes in the tubules varied in different portions of the tubule. It is obvious that the size of the dose and the

length of survival time both play a factor in the tubular damage.

Toadfish—8 cc./kg., killed 3 days after injection.

Tubules were dilated—otherwise normal in appearance. Nuclei appeared slightly large. Brush border was not visible.

Tautog—8 cc./kg., killed 3 days after injection.

Second part of proximal tubules showed all changes from dilatation without other severe damage to complete necrosis of cells and loss of architecture of tubules. Severe hemorrhage into tissues was found in one instance.

Toadfish—8 cc./kg., killed 4 days after injection.

Tubules were widely dilated, with low cuboidal epithelium, pale swollen nuclei. In some cases nuclei were small and pyknotic or faded completely from the cells.. Some debris was present in lumen. In some sections damage was only slight, cell membrane and brush borders being clear. Nuclei were normal in appearance. In one section damage of epithelium was rather severe.

Tautog—8 cc./kg., killed 4 days after injection.

Second part of proximal tubules were widely dilated. Epithelium was low cuboidal, nuclei pale and swollen, cell membranes were not visible, and cytoplasm was scant. Much debris was found in lumen. In some tubules, there was destruction of cells. Vacuoles were present in some cells. In some sections, the second part of proximal tubules showed complete destruction of cells and loss of architecture of tubules.

Toadfish—8 cc./kg., killed 7 days after injection.

Some destruction of cells—most of nuclei were pale and enlarged. Debris was present in lumen. (Fig. 8, Plate 1.) (Fig. 2, Plate 2.)

Tautog—8 cc./kg., killed 7 days after injection.

In second part of proximal tubules large vacuoles appeared in cells, often with destruction of architecture of tubule. (Fig. 6, Plate 1.) Nuclei were pale and enlarged. Some destruction of cells was present with rupture of cell membrane after vacuolation. In second part of proximal segment there was more change from normal than in toadfish sections (Fig. 2, Plate 3).

Toadfish—16 cc./kg., killed 4 days after injection.

Vacuoles were present in some cells, nuclei swollen, pale, and with prominent nucleoli; cell membranes were indistinct. Debris was found in lumen; tubules were widely dilated. No brush border was apparent. The collecting tubules appeared the same as the convoluted tubules. In some sections brush border was still present, tubules dilated, nuclei swollen and pale, cells low cuboidal. There was no obvious destruction of cells—cell membranes intact.

Tautog—16 cc./kg., killed 4 days after injection.

The second part of the proximal tubules showed complete destruction of cells, and the architecture of tubule was lost in many cases. In other tubules there were dilated, pale, swollen nuclei with prominent nucleoli. The cells were low cuboidal and tubules were widely dilated. The larger collecting tubules often showed extensive damage to epithelium; others appeared normal except for low cuboidal epithelium. There was some hemorrhage into tissue in some sections. A comparison of these effects is shown in tabular form in Table I.

TABLE I.

Toadfish	*Tautog*
8 cc./kgm. 3 days	
Tubules dilated—otherwise normal.	Dilatation—> complete necrosis of cells and loss of architecture of tubules. One case of hemorrhage into tissues.
8 cc./kgm. 4 days	
Tubules widely dilated, some nuclei small and pyknotic or faded from cell. Debris in lumen. Many sections only very slightly damaged.	Tubules widely dilated. Nuclei pale and swollen. Cell membranes not visible and cytoplasm scant. Much debris in lumen. Some sections show: destruction of cells; vacuoles in cells; complete destruction of cells and loss of architecture of tubules.
8 cc./kgm. 7 days	
Some destruction of cells: most nuclei pale and enlarged. Debris in lumen.	Large vacuoles in cells often with destruction of architecture of tubule. Some cells show rupture of cell membranes after vacuolation.
16 cc./kgm. 4 days	
Vacuoles present in some cells: nuclei pale, swollen and with prominent nucleoli: cell membranes indistinct. Debris in lumen. In some brush border still intact. No obvious destruction of cells. Cell membranes intact.	Tubules show complete destruction of cells and architecture of tubules lost in many cases. In others there are pale, swollen nuclei with prominent nucleoli. Tubules widely dilated. Hemorrhage into tissues in some cases.

EFFECT OF QUITENINE ON KIDNEY TUBULES

PLATE I

Explanation of Figures

Tautoga onitis (Tautog)

Camera lucida drawings X 650

1. Malpighian body, neck and proximal segments of tubule. (Normal)
2. Cross section of neck segment of tubule (Normal)
3. Cross section of first portion of proximal segment of tubule (Normal)
4. Cross section of collecting duct (Normal)

4a. Cross section of second portion of proximal segment of tubule (Normal)

5. Malpighian body, neck and proximal segments of tubule (Pathological) 8cc./Kgm. 7 days
6. Cross sections of second portion of proximal segments showing vacuoles (Pathological) 8 cc/Kgm. 7 days
7. Cross section of collecting duct (Pathological) 8 cc./Kgm. 7 days

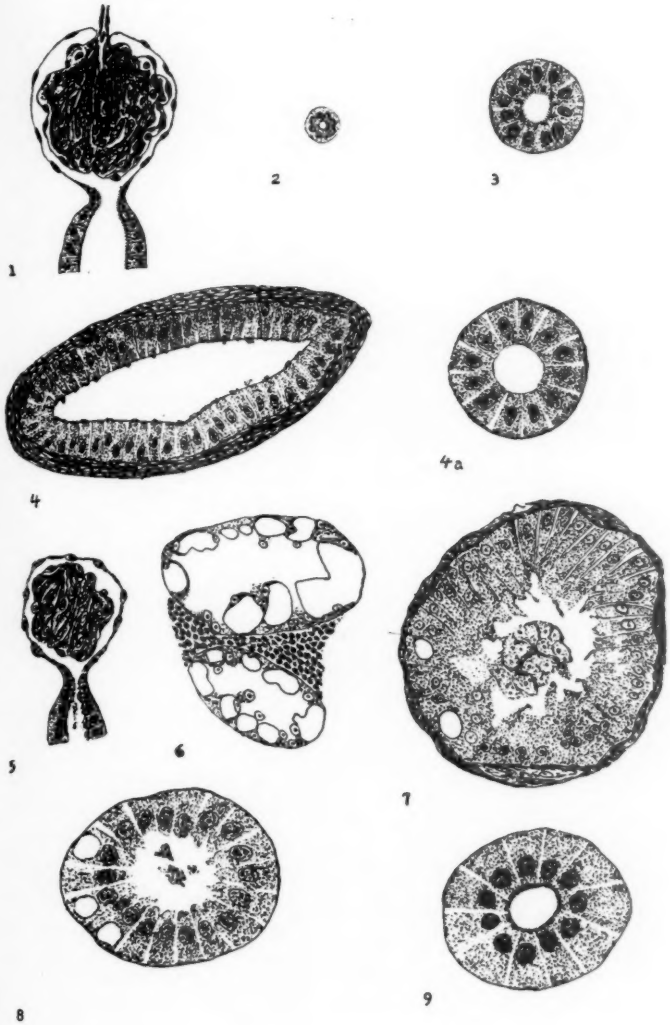
Opsanus tau (Toadfish)

Camera lucida drawings X 750

8. Cross section of tubule (Pathological) 8 cc./Kgm. 7 days
9. Cross section of tubule (Normal)

PLATE I

KIDNEY OF TAUTOGA ONITIS AND OPSANUS TAU



EFFECT OF QUITENINE ON KIDNEY TUBULES

PLATE II

Explanation of Figures

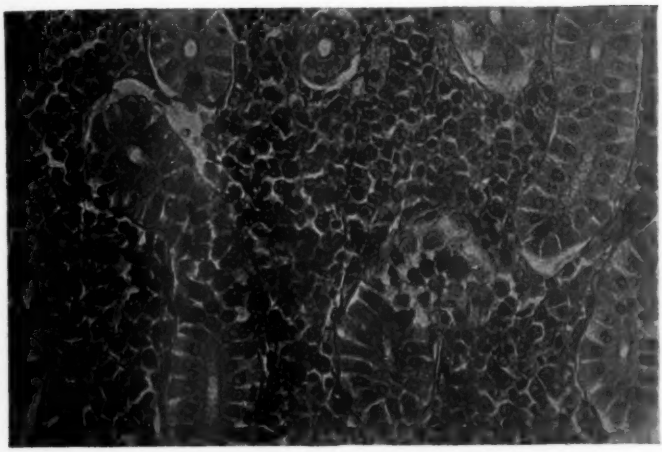
Opsanus tau (Toadfish)

Microphotographs of Kidney X 300

1. (Normal) Hematoxylin and Eosin
2. (Pathological) Iron Hematoxylin. 8 cc./Kgm. 7 days

PLATE II

KIDNEY OF OPSANUS TAU



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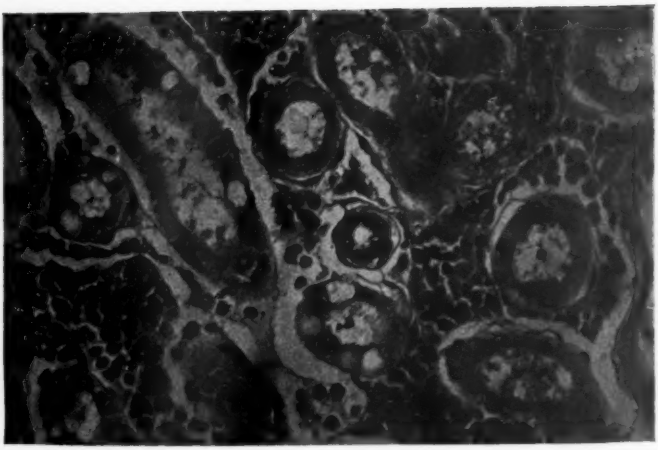


2

EFFECT OF QUITENINE ON KIDNEY TUBULES

PLATE III

Explanation of Figures

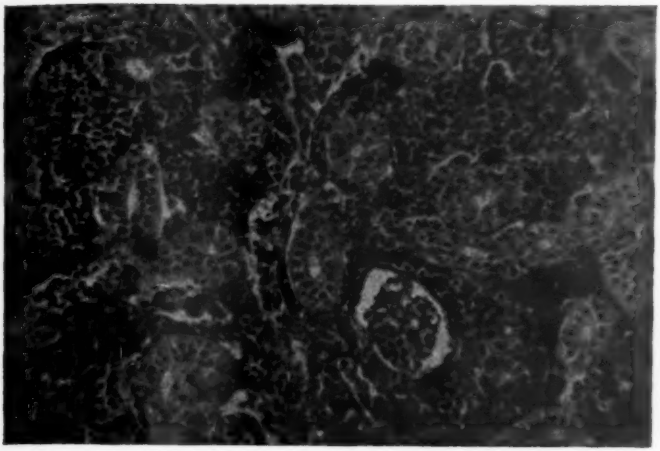
Tautoga onitis (Tautog)

Microphotographs of Kidney X 450

1. (Normal) Iron Hematoxylin
2. (Pathological) Iron Hematoxylin. 8cc./Kgm. 7 days

PLATE III

KIDNEY OF TAUTOGA ONITIS



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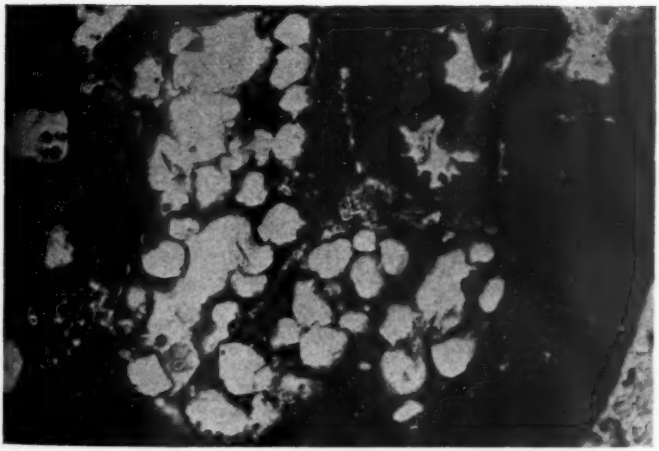

2

Conclusions

It is likely that the difference in the method of excretion of the drug accounts for the difference in its destructive action on the tubules of the glomerular and aglomerular fish. It is obvious that for the drug to be excreted by the aglomerular kidney, it must pass through the walls of the tubule into the lumen, probably by being secreted by the tubules. While this mechanism is also conceivable in the glomerular fish, it is not the only way that quitenine can get into the lumen of the tubules of the glomerular fish. It is possible that the drug may pass through the capsule and then be concentrated in the tubules by the reabsorption of water by the tubules, thus having a much higher concentration of the drug in the tubules of the glomerular kidney than in the tubules of the aglomerular kidney.

This damage may have been brought about in two ways: the drug has a very strong destructive action on all tissues, and it may cause the destruction of the tubular epithelium by its necrotic action, a purely chemical effect; or it is conceivable that the drug may have become so concentrated in the tubules that it had crystallized and caused mechanical damage in its passage through the tubules. (The fact that no crystals of quitenine were found in the section does not necessarily disprove this theory, for the fixing fluids and alcohol would dissolve out the quitenine).

It may be that the damage to the tubules is a combined chemical and mechanical effect—the damage in the first part of the proximal tubule being just chemical, while in the second part of the proximal tubule and collecting tubules, where the urine would be more concentrated, it may be both chemical and mechanical.

SUMMARY

1. A description of the tubules of the tautog is given.

2. The action of quitenine on the kidneys of tautogs and toadfish is described and compared.

3. The action of quitenine on the glomerular kidney is apparently more severe than in the aglomerular kidney.

We are indebted to Miss Flora Thompson and Dr. Henry W. Poetter for their assistance in some of the technical phases of this work.

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ANTERIOR TRANSVERSE DURAL LIGAMENTS: THEIR INCIDENCE, DISTRIBUTION, AND RELATION TO THE STRUCTURE OF THE DURA

WM. D. SEYBOLD*

The finding of thread-like transverse bands in the dural sac extending across the ventral surface of the spinal cord in an adult human subject, prompted our search for similar structures in all available material. A report of the findings in 133 adults and 4 fetuses has been previously published (Seybold, 1940). Since that time we have had the opportunity to extend our observations by examining an additional 30 adult and 75 fetal specimens. The findings in the fetal group have an important bearing on the question of whether or not these structures are "normal," *i.e.*, developmental or pathological, and hence occasion the present report.

The anterior transverse dural ligaments are slender but strong bands of dense fibrous tissue extending through the arachnoidea on the ventral surface of the cord and attached by their extremities to the inner surface of the dura somewhat anterior to the attachments of the teeth of the denticulate ligaments (fig. 1). Their histological characteristics have been presented in detail in the previous paper, and it has been shown that they are an isolated portion of the dura and merely ensheathed by the arachnoidea rather than a part of it. Similar structures, differing only in size, are found in the fetus (fig. 2).

Examination of the fetal specimens was carried out under the magnification afforded by a reading glass in 50 specimens and under dissecting binoculars in the remaining 25. In addition, whole mounts of the ligament and surrounding arachnoidea and microscopic preparations from the fetus were stained and studied. The fetal structures are counterparts of the adult ligaments except in size.

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EXPLANATION OF FIGURES

1. Ventral aspect of an adult cord showing anterior transverse dural ligaments.

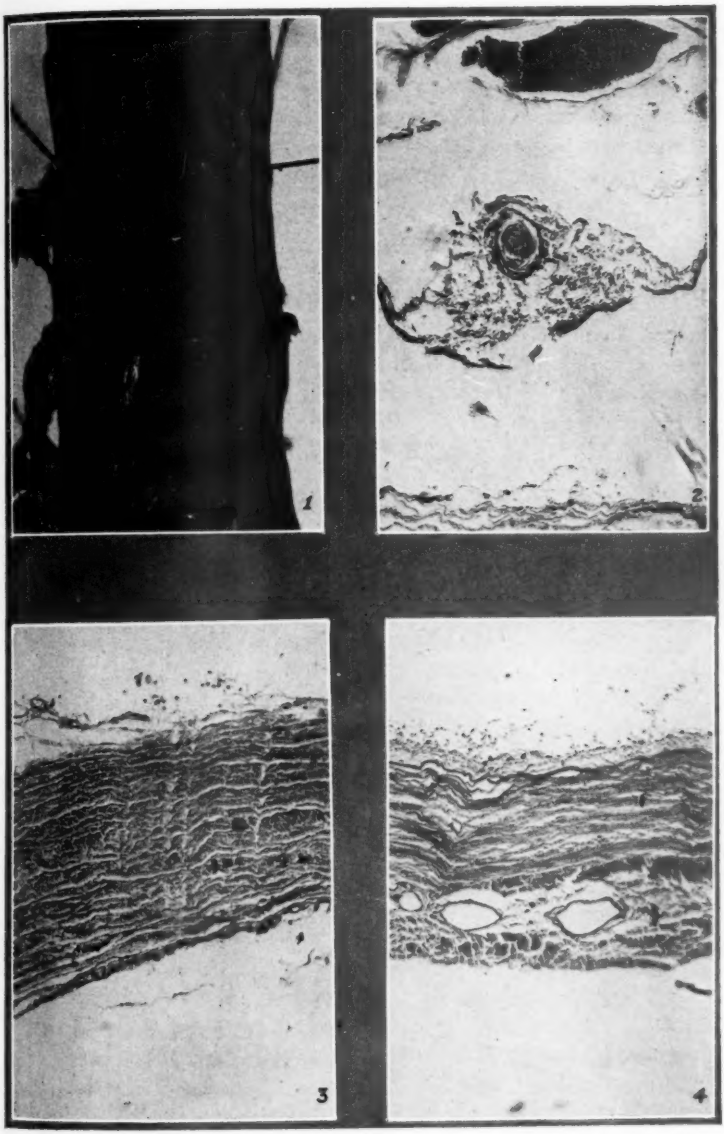
2. Cross-section of ligament from a full-term fetus showing it embedded in the arachnoid membrane. The innermost portion of the dura can be seen in the lower portion of the field, and the blood vessel in the upper part of the field is closely applied to the ventral surface of the cord.

3. Transverse section through the dorsal portion of the spinal dural sac. The dense collagenous bundles are, for the most part, cut at right angles to their long axis.

4. Transverse section through the ventral portion of the spinal dural sac. The two strata, an outer longitudinal and an inner transverse, are plainly evident.

Of the 113 adult specimens in the first group studied, one or more anterior transverse dural ligaments were found in 80 or 70.8 per cent. In the second series of 30 adult subjects, they were found in 27, or 88 per cent. This discrepancy is accounted for by the fact that the spinal dural sac in the last series was intact until opened for this study; while the specimens used for the previous report had been the object of previous class dissection, and in many instances there had been extensive destruction of the meninges and the cord itself. In a total of 143 specimens examined, the incidence was 75 per cent. In the fetal group the bands were observed in 50 specimens, 66.6 per cent. In the 25 of this fetal group, dural bands were noted in 21 or 84 per cent. These were examined under dissecting binoculars at a magnification of 25 diameters rather than with the reading glass used for the first 50. There are no apparent differences in incidence based on age, sex, or race. Fetuses of the fifth month, the youngest examined, as well as individuals of 85 years presented these structures.

There is a correspondingly close agreement in the regional distribution of the structures in the fetal and adult series. Of the 30 adult cords (which for reasons given above were best suited for our purposes) in the second series, 27 were found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to have one or more of these ligaments. In these 27, ligaments were found in the thoracic region in all, 100 per cent; in the cervical region in 30 per cent; and in the lumbosacral and caudal regions in 37 per cent. Of the 25 fetal specimens examined under binoculars, 21 presented one to several ligaments. Thoracic bands were found in all 21, 100 per cent; cervical in 20 per cent; and lumbosacral and caudal in 20 per cent. (Table I.)

Discussion

The most satisfactory explanation for the presence of these structures is one based on the developmental concept that the meninges arise as condensations in an early, undifferentiated mesenchymal mesh surrounding the primitive nervous system. Weed (1932) says: "The mesodermal cellular layer, closely applied to the nervous system and two or three cells in thickness in the earliest stages, gradually in the course of embryonic

Table I.

Anterior transverse ligaments of the dura

Incidence

	No. Specimens Examined	No. Containing Ligaments Per Cent
Adult	143	107 (75)
Fetal	75	50 (66)

Distribution in Specimens Containing Ligaments

	Cervical Per Cent	Thoracic Per Cent	Lumbosacral Per Cent
Adult	30	100	37
Fetal	20	100	20

growth formed a definitive membrane, the pia mater, still intimately investing the nervous structures. The arachnoid membrane developed, in conjunction with the inner portion of the dura mater, out of the secondary condensation, which was first noted between the cranial blastema and the primative pial aggregation of cells. The final differentiation and separation of the arachnoid membrane from the inner surface of the dura mater were later processes; but at the stage of about 50 mm. in the pig embryo the separation was largely achieved as demonstrated by the possibility of obtaining silver

preparations of the inner surface of the dura mater showing a polygonal type of cell." During the "final differentiation and separation of the arachnoid membrane from the inner surface of the dura mater" *(loc. cit.)* the ligamentous structures in question could conceivably arise; and their persistence is a reminder of the intimacy of the two membranes during the course of their development. In birds (Hansen-Pruss, 1923; Kappers, 1926) and Ambylstoma punctatum (Flexner, 1929) points of actual fusion of the dura and arachnoidea are found to persist in adult life, though complete separation is the rule in higher mammals.

An explanation of the developmental forces concerned in the production of these structures will not be attempted, but the existence of an inner layer of transverse fibers in the ventral portion of the spinal dural sac may account for their presence on the ventral surface exclusively. The dorsal wall of the sac can be shown both by gross dissection and microscopic examination to be composed almost entirely of heavy collagenous bundles running longitudinally (fig. 3), while the ventral portion consists of an outer layer of longitudinal fibers and an inner layer of transverse fibers lying immediately external to the mesothelial lining (fig. 4).

The parallel incidence and distribution in the fetal and adult groups, the peculiar organization of the ligaments, and the otherwise normal appearance of the meninges militates against an inflammatory origin. They may appear in response to tension on nerve roots, but such an explanation is rendered very doubtful on the grounds that they do not necessarily occur at the level of exit of the nerve roots and because they are more commonly found in the thoracic portion of the sac rather than in the regions of the limb plexuses.

The possibility exists that the ligaments may be the result of thrombosed and subsequently organized vessels running a transverse course through the arachnoidea, but no such vessels have thus far been demonstrated in any of our material.

Any phylogenetic significance that may be attached to these structures has been previously considered in the light of our negative findings in dogs, cats, and a baboon. Whether or not

they occur in the higher anthropoids remains to be demonstrated.

Summary

1. Anterior transverse fibrous bands, extending from one side of the ventral portion of the spinal dural sac to the other and traversing the arachnoid membrane, were found in 75 per cent of 143 adult human subjects and in 66 per cent of 75 fetal specimens examined.

2. In both adult and fetal groups they occur most commonly in the thoracic region of the dural sac.

3. The ligaments in the fetus are counterparts of those of the adult except in size.

4. The collagenous fibers composing the dorsal portion of the spinal dural sac have been shown to be disposed almost exclusively in a longitudinal direction while the ventral portion of the sac is composed of two strata, an outer longitudinal and an inner transverse layer of fibers.

5. Evidence has been adduced to establish the anterior transverse ligaments of the dura as true anatomical or "normal" rather than pathological structures.

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THE USE OF RADIO-ACTIVE PHOSPHOROUS IN THE TREATMENT OF LEUKEMIA

CAROLINE WEBSTER*

Leukemia presumably arises more or less focally, as in the marrow or lymph nodes, and only subsequently progresses to involve other tissues than those normally concerned in the production of leukocytes. Nevertheless, as observed clinically in the human subject, it is invariably a generalized disease. Thus it is not at present conceivable that any method of regional treatment can do more than palliate by causing regression of the main sympton-producing foci.

Recognizing the fallacy of trying to control leukemia, a generalized disease, by local irradiation, numerous workers have sought to treat the disease *in toto* by so-called spray irradiation of large segments of the body or by total irradiation. While notable palliations are obtained with this method, the obvious limitation lies in the fact that the patient's tolerance to whole body irradiation is nowhere near sufficiently great to permit taking full advantage of the differential in sensitivity between leukemic and normal tissues.

If, then, it became possible to deliver radiant energy in considerably higher concentrations to the leukemic tissues which are usually more radiosensitive than the normal cells, the damage done to the leukemic tissues would be greater than to the normal tissues, and therefore we might approach a more nearly ideal method of treatment of leukemia by irradiation.

EFFECTS OF RADIO-ACTIVE PHOSPHOROUS

In July, 1940, J. H. Lawrence first reported on the use of radio-active phosphorous in treating leukemia.

Since the invention of the cyclotron by E. Lawrence, at Berkeley, many radio-active substances have been made available for experimental and clinical study. Radio-active phosphorous,

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whose atomic weight is 32, is produced by the bombardment of ordinary red phosphorous, whose atomic weight is 31, by very rapidly moving deuterons generated in the cyclotron. The resulting material, converted into an aqueous solution of dibasic sodium phosphate, retains all properties of ordinary phosphate with addition of radio-activity.

As radio-activated phosphorous is handled by the animal organism like ordinary phosphorous, the distribution is never equal. It is quickly concentrated in bone-marrow, liver, spleen, bone, and lymph nodes. Also, there is a constant breakdown and decay. Erf and Kenan (1942) made a study of the retention of radio-active phosphorous by tissues of patients dead with leukemia. They found that, in patients who had received radio-active phosphorous many days before death, the concentration of the radio-active phosphorous was frequently greatest in the osseous tissues, while in those who had received radio-active phosphorous just before death, the concentrations were greatest in such tissues as the bone marrow, lymph nodes, spleen, and liver. These findings suggest that radio-active phosphorous is first utilized by the more rapidly metabolizing tissue, or those most frequently infiltrated with leukemic cells, and that later it finds its way to the bones.

Radioautographs (made by placing the cut surface of a specimen of tissue, under refrigeration, against an x-ray film for 36 hours) demonstrate that even in a given tissue the distribution of the radiation is not uniform. Also, since the phosphorous atoms within the body shift continuously, because of metabolic processes, the level of activity found in the tissue at one time may not prevail at any other time.

Studies in animals (Low-Beer, Lawrence, and Stone, 1942) showed that in animals with leukemia, lymphosarcoma, lymphoma, and other tumors, a higher percentage of phosphorous is taken up in the pathological tissue than in any of the normal tissues with the exception of bone. Whenever the rate of the cell formation is increased, the uptake of phosphorous is also increased markedly. Therapeutic doses of radio-active phosphorous reduce the number of white cells in the peripheral blood of leukemic animals to a greater extent than in that of normal animals or those with localized tumors.

Studies on patients who were given tracer doses of radio-active phosphorous showed that wherever leukemic or neoplastic infiltration existed the uptake of phosphorous was high.

According to Erf, Kenan, and Tuttle (1942) in a study of phosphorous metabolism of the blood in patients with leukemia and polycythemia, of a quantity of phosphorous ingested by humans, various amounts are absorbed and excreted by the circulating red blood cells, with the peak of exchange occurring between 12 and 24 hours after consumption. A similar exchange takes place in the circulating white blood cells, which, however, retain phosphorous in the phospholipid and nucleoprotein fraction for much longer periods and in greater quantity than the surrounding red blood cells. Plasma also has a specific rate of phosphorous exchange. The characteristic trends of the exchange of phosphorous in various fractions of red blood cells, white blood cells, and plasma were similar in four patients in spite of the different disease processes involved.

Articially radio-activated elements emit rays which are similar to those given off by radium and other naturally radio-active elements, so the artificially activated elements may be used as a substitute for radium.

Radio-active phosphorous has a half-life of 14.3 days as compared to radium and thorium which have half-lives of hundreds of years. Thus with radio-active phosphorous there is no danger of cumulative radiation effects. Nearly 50 per cent of any given dose is normally excreted during the first six days and excretion continues thereafter. After six weeks no significant amount of radiation can be found in any tissue following a single administration of radio-active phosphorous.

It is evident that the advantages of using radio-active phosphorous are its half-life of 14.3 days, that it emits only beta rays, that it localizes in rapidly growing tissues, and it may be manufactured as desired.

Administration

The material, in solution, may be administered orally or parenterally. Single oral doses have varied from 1 to twenty

millicuries, single intravenous doses vary from 0.2 to 2 millicuries. For oral administration the dose is mixed with equal parts of orange juice and is taken before breakfast.

Too much of the phosphate may result in a diarrhea, but the proper dose is pleasant to take and free from untoward reactions. Repeated small doses, at an interval of one to three times a week, are preferred to a single large dose.

The preference for continued small doses is based on the facts that cells are known to be more radiosensitive at one stage of the mitotic cycle than at any other, and all cells do not reach this stage at the same time; hence, continuous irradiation may affect more cells than interval irradiation even when the level of continued irradiation is lower than the peak of the interval method. The amount retained in the body from a given total amount is higher when the total amount is administered in small fractions. The utilization of phosphorous depends upon the need of tissue cells for this metabolite; this need is greatest at the time of mitosis. The body is incapable of using more phosphorous at one time than its daily requirements.

After the last administration of radio-active phosphorous, some biologically active radiation will remain in the body for 25 days or more. When the white count falls to about 30,000 the intervals between treatments should be lengthened and the trend of the count observed. If the leukocyte count reaches a reasonably constant level above 10,000, one or two additional doses of the same size as those used to maintain the previous radiation level will usually suffice to bring the count down to normal.

Clinical Results

To date the results of the use of radio-active phosphorous in treating leukemia are incomplete. Only a small percentage of the cases have been treated with radio-active phosphorous, and sufficient time has not elapsed in which to judge the actual results. Craver stated that seven out of nine patients with chronic myelogenous leukemia showed clinical and hematological improvement; that in a rather unfavorable group of

eleven cases of chronic lymphatic leukemia, four had shown a good response by regression of spleen and lymph nodes, without much effect on the blood or marrow; but that in fifteen cases of acute leukemia thirteen had shown no evidence of favorable response.

Fitz-Hugh and Hodes reported five cases of chronic myelogenous leukemia, two of which obtained remissions and three of which were far advanced to begin with; four cases of chronic lymphatic leukemia, two of which obtained remissions, one was far advanced, and one had just started on the therapy; and four cases of acute leukemia in which the treatment was apparently of no value.

Although one cannot draw hasty conclusions, it is evident that this new method of treating leukemia is based upon good theory and sound experimental data; and, although the clinical results are as yet incomplete, they are indeed promising, with the exception of the acute leukemias whose management apparently still remains to be discovered.

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RORSCHACH METHOD OF PERSONALITY AND INTELLIGENCE EVALUATION

R. B. WHITE*

The use of ink blots for psychological investigation is not a recent innovation as Klopfer and Kelly (1942) have pointed out. Justinius Kerner observed that these blots might take on varied and bizarre meanings as he observed them. Binet first suggested their use in the study of personality in 1895, but it remained for an American, Whipple, to publish the first standard set of blots for psychological procedures. These early workers were primarily interested in the use of ink blots stimuli for free associations rather than as a standardized and scorable test procedure.

Herman Rorschach (1921) published the results of ten years of work with a series of ten ink blot pictures which he had arbitrarily selected from among several thousand trial blots as being best suited for use as stimulus material in his diagnostic procedure. At the same time he shifted the interest from the associative or imaginative aspects of the subject's response to his cards to the manner in which the stimulus material was handled. Klopfer and Kelly (1942) summed this up by the statement: "The interplay between the structural characteristics of the stimulus material and the personality structure of the subject is reflected in certain formal categories which describe characteristics of the concept formations, and which *Psychodiagnostik* established as the basis of an objective method for personality study."

The Rorschach method is concisely set forth in the original *Psychodiagnostik* and has remained essentially unchanged to the present time which is truly a tribute to the genius of Herman Rorschach. Apparatus consists only in the ten cards each of which has a bilaterally symmetrical and irregular ink blot on it. Five blots are colored and five are not. The procedure of the test has no rules or limitations to which the subject must adhere and consists in merely presenting the

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cards to him one at the time with the question, "What might this be?" No further explanation is necessary and any questions that the subject may ask can be answered with the statement, "That is entirely up to you." Any answers, comments, or exclamations that he makes are recorded, as is the length of time taken on each card and time elapsing between his first look at the card and first answer (reaction time). When he is through with one card, it is laid aside and another one handed to him.

The factors in the results that are of interpretive significance are several in number. First is the number of responses. Normal subjects give from fifteen to thirty answers; whereas depressed individuals give fewer, and euphoric or manics give more. Next is the time element. The total time consumed with the test is usually about thirty minutes and the usual reaction time is fifteen to forty-five seconds for each card. Failure to give an answer to a card is rare in normal individuals.

The three principal factors to be considered in evaluating a response are: (1) Location of the response. That is whether the whole blot or some large or small portion of it is used. (2) The determinant of the response. In other words, what quality or characteristic of the blot brought that particular answer used into the subject's mind—whether the form, color, or appearance of human or animal in motion. (3) The content of the response: whether the blot is seen as in animal, human, man-made object, anatomy, or landscape. The answers are then scored on a prepared score sheet and tabulated in a prescribed manner. The exact technique would require too lengthy a discussion to be set forth here. The symbols and abbrevations used are listed in the sheet attached to this paper.

To the trained operator, the results give a graphic account of the dynamics of the subject's entire personality. Briefly, the principles of interpretation of the responses are as follows:

1. *Location.* The manner in which the subject approaches the problem of interpreting the ink blot reflects the manner in which he approaches his daily life situations. A meticulous and orderly person approaches each card in the same

orderly fashion, and tends first to use the whole card in a concept, then to use some large detail, and, finally, to pick out the small details.

2. *Form Responses.* The number and quality of responses using shape as the determinant reflect the balance between logical reasoning and emotional impulsiveness. The greater the use of pure form responses, the more the individual tends to be cold and calculating rather than emotional.

3. *Movement Responses.* The use of movement responses reflects the use of imagination and original ideas and the lack of this type of answer indicated paucity of these creative qualities.

4. *Color Responses.* These deviate from the form responses in the opposite direction from that taken by movement answers. Both indicate a higher degree of spontaneity than form responses; but action responses point toward promptings from within and the spontaneity reflected in color responses show reaction to responses from without. The use of color corresponds to the subject's relationship to the outer world, especially the emotional aspects of the relation. The neurotic personality carrying its higher emotional charge reacts more violently to the colored cards and often refuses to respond to them at all (color shock). It is interesting to note that, as Brosin and Fromm (1940) concluded, color blindness in no way affects the subject's reaction to the colored cards. Nor does the presence of a color vision defect produce any abnormal reactions by virtue of a knowledge of the defect on the part of the patient and a resulting greater concern on his part when the colored cards are encountered. The neurotic subject with normal color vision who claims to be color blind will show definite color shock on the test.

5. *Shading Effects.* These are the newest of the determinants to be introduced and their use indicates anxieties of a vague and undifferentiated nature.

This rather hasty discussion of interpretive significance of response types is by no means complete and interpretation of the record as a whole has not been touched upon. Any tendency in a record must be evaluated in terms of the other forces revealed by the examination. In other words, the final con-

clusion drawn from a Rorschach record is a resultant rather than a summation of individual factors. Nor have the psychotic manifestations been considered. For a more detailed picture of normal and abnormal records the reader is referred to Table I, taken from the original *Psychodiagnostik*.

According to Klopfer and Kelly (1942), the principal information ascertained by the Rorschach tests are: (1) the degree and mode of control with which the subject tries to evaluate his experiences and actions; (2) the responsiveness of his emotional energies to stimulations from outside and promptings from within; (3) his mental approach to given problems and situations; (4) his creative or imaginative capacities, and the use he makes of them; (5) a general estimate of his intellectual level and the major qualitative features of his thinking; (6) a general estimate of the degree of security or anxiety, of balance in general, and specific unbalances, and (7) the relative degree of maturity in the total personality development.

The most important aspect of Rorschach examination is the fact that the subject has no way of knowing upon what basis he is being tested or for that matter that he is being "tested" at all. Frank (1939) emphasized this point: "The Rorschach method offers a procedure through which the individual is induced to reveal his private world by telling what he sees in the several cards upon which he may project his meanings, significance, and feelings, just because they are not socially standardized objects or situations to which he must give culturally prescribed responses. The Rorschach method is essentially a procedure for revealing the personality of the individual as an individual, as contrasted with rating or assessing him in terms of his likeness or conformity to social norms of action and speech. It is just because the subject is not aware of what he is telling and has no cultural norms behind which to hide himself that the Rorschach method is so revealing."

The uses of the Rorschach procedure are many and are by no means limited to the field of pure psychiatry. The last world war saw the utility of the intelligence and aptitude tests firmly established, and it is not unlikely that the present

TABLE I.

General Distribution of Rorschach Test Scores.

Subjects	F Per Cent	M Per Cent	C	W	Apper. Type	A Per Cent	Original* Per Cent
Artists	90–100	5+	1–2FC, 2–3CF, 1–2C	10+	W–(D–Dd)	10–20	30–50
Intelligent	80–100	5+	1–3FC, 1–2CF, O C	7–10	W–D	20–35	20–30
Av. Intelligence	70–80	2–4	1–2 FC, 0–1 CF, O C	4–7	W–D	30–55	0–20
Unintelligent	60–70	0–2	1–3 FC, 1–3 CF, O–1C	3–4	DW–D	50–70	0–20
Depressed Mood	80–100	0–2	OFC, O CF, O C	0–3	D–Do	60–80	0–10
Manic Mood	60–70	3–5	1–2 CF, 2–3 FC, 1–2 C	8–10	W–D–Dd	40–50	20–30

LIST OF SYMBOLS AND ABBREVIATIONS

R—Total Responses.
W—Plate Interpreted as a Whole (Whole Answer).
DW—Plate is interpreted as a whole secondarily, the answer based primarily on a detail.
D—A normal detail of the plate (Detail Answer).
Dd—An unusual or small detail (Small Detail Answer).
S—White intermediate figures (Space Detail).
Do—A detail is interpreted in the place of a whole.
Apper.—Apperceptive Type. Relation of the apperceptive modes, W, D, etc.
Sequence—Sequence of W, D, etc., in the individual plate.
F—Form Answers. Interpretation is determined primarily by the form of the blot.
M—Movement Answer. Interpretation is determined by kinaesthetic influences.
FC—Form-Color Answers. The interpretation is determined primarily by the form and secondarily by the color of the blot.
CF—Color-Form Answer. The interpretation is determined primarily by the color, secondarily by form of the blot.
C—Color Answer. The interpretation is determined by the color of the blot alone.
H—Interpretation of Human Figure.
A—Interpretation of Animal Figure.
Hd—Interpretation of Part of the Human Figure.
Ad—Interpretation of Part of Animal Figure.
Obj.—Interpretation of Inanimate Object.
Ldscp.—Interpretation of Landscape.

A Per Cent—Animal Per cent. $\frac{A + Ad}{R}$ X 100.

Orig. Per Cent—Per Cent Original Answers (Answers occurring no more than once in 100 Tests) of Total Answers.

NOTE: Above symbols are from Rorschach's original publications. Since that time movement answers have been divided into M (Figures in human-like action), and FM (Animals in animal-like action). In addition K (Shading as diffusion such as smoke or clouds) has been added.

global conflict will see the use of the Rorschach test spread into many branches of industry and medicine.

Brussel and Hitch (1942) pointed out that the Rorschach test is useful in the psychiatric and psychological examinations of both normal and abnormal personalities. They go on to state that the German military authorities have used Rorschach testings for several years in the selection of troops for special posts such as aviation, intelligence, and parachute combat, where it is of the utmost importance that the men selected be quick-witted, reliable, steady, and calm under stress. Harrower-Erickson (1940) lists the major military uses of the Rorschach method as being the weeding out of emotionally unstable men from positions of responsibility, supplementing intelligence test scores, differentiating between the malingerer and the individual with a genuine functional or organic disease, and diagnosing "shell shock." It is quite obvious that to measure an inductee's or officer candidate's isolated mental abilities and to more or less ignore the role of his emotional liability, impulsiveness, and order of thinking processes is at best unsatisfactory. The ideal test for the selection of personnel for positions of responsibility must be one which merges intelligence and personality into a single characterological unit.

Bigelow (1940) has shown the need for a standardized method of determining the aptitude of candidates for flight training not only from the standpoint of intelligence, physical condition, mechanical ability, and motor coördination, but also from the point of how the individual will react to the many tension situations to which this training will necessarily subject him. Billig (1943) states ninety per cent of failures to complete flight training satisfactorily is due to emotional maladjustment. The vital question of whether or not the candidate will allow emotion to take over in a moment of crisis when his and other lives and equipment will depend on his cool judgment should be determined before such a situation will arise. A series of several thousand Rorschachs on flight candidates with appropriate checks on the actual performance of these individuals would allow proper criterion to be set up for the evaluation of aviation aptitude by examiners with less formidable psychiatric background than is now required. But

as Ross (1941) has shown, the Rorschach can be properly interpreted only by viewing both the person and the record obtained from him as a whole and not by reliance on isolated findings in the Rorschach record. But Munroe (1942) has demonstrated a close correlation between actual performance and adjustment as determined by her rapid inspection diagnosis of 100 Rorschach records of freshmen women college students. This bears out Bigelow's suggestion that records might be satisfactorily interpreted by technicians rather than Rorschach experts. At least such a plan would serve as an excellent screening test and borderline cases could be referred to more competent men for a final decision.

Harrow-Erickson (1941) has devised a procedure for administering the test to groups of as many as fifty men at one time by use of projection of color slides of the cards on a screen. While not as exact as the individual test, the results are valid enough to warrant its use in selected instances.

In summary, it may be stated that the Rorschach method is unique in that it reveals to an amazing extent the emotional, intellectual, and creative drives present in an individual's personality in one composite and dynamic picture. Its utility and practicability are unquestionable and its use has steadily grown in this country since its introduction by Levy in 1923. And finally, the impetus given work in this field by the present war will undoubtedly promulgate a wide application of the test in medicine, industry, and military service in the future.

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SULFONAMIDE CRYSTALS APPLIED DIRECTLY TO FIBROBLASTS AND LEUCOCYTES *IN VITRO**

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There is a growing appreciation among clinicians of the value of direct applications of sulfonamide crystals to wounds and to the body cavities. The present difficulty, however, seems to be one of discriminating and choosing the drug which is most effective and safe (Fraser). Notable steps have been taken in the experimental study of this problem. Sulfonamide crystals have been dusted into clean and infected wounds produced in laboratory animals. Crystals applied to clean wounds have been studied with respect to the brain by Russell and Falconer and also by Hurteau; to the eye by P'an; the peritoneum by Throckmorton; joints, muscle as well as to the pleural and peritoneal cavity, by Key; while reactions in other clean wounds are reported by Goodwin and by Hawking. The value of crystalline applications to wounds experimentally contaminated with organisms related to the production of gas gangrene has been studied by Hawking and by Reed.

In a thoughtful and technically brilliant study, one of the groups in Cambridge, England, which has perfected tissue culture technique as a tool for the analysis of biochemical problems, recently reported on the toxicity of several sulfonamide compounds when applied directly to cell cultured *in vitro* (Jacoby, Medawar and Willmer). These authors point out that even if embryonic tissues commonly employed for cultures *in vitro* have a relatively high resistance to inhibitory agents, they permit, nevertheless, an excellent opportunity for carefully controlled and detailed observations of drug effects on living cells not favored by a circulatory system or the activities of detoxifying mechanisms. Careful inspection of the results obtained by Jacoby, Medawar and Willmer should

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bring confidence and guidance to the surgeon faced with selecting sulfonamides for direct application to tissue surfaces.

The present study, launched before the results of the Cambridge group were available, was designed to observe the reactions of cells to the presence of sulfonamide crystals. In contrast to the work of that group the present authors were concerned with the direct application on the clotted tissue culture medium of crystals not previously dissolved in either serum, Tyrode's or Ringer's solution. There were several differences in the culture methods employed. Results are reported for three compounds not used by the English workers; namely, sulfabenamide, sulfaguanidine, and sodium sulfadiazine. Finally, all compounds used in the present investigation were also applied to spleen fragments in order to discover whether the characteristic migration of leucocytes from this organ would be affected.

Material and Methods

The medium for all cultures used consisted of rooster plasma and an extract derived from chick embryos incubated for 7 to 10 days and made up to 20 per cent in Tyrode's solution. Hanging drop preparations were used for all four types of explants (*c.f.*, Cameron).

(1) *Freshly explanted fragments from the ventricle of chick embryos incubated for 8–10 days.* As soon as the plasma clot had become solidified in such preparations, crystals of the sulfonamide under study were dusted directly onto one side of the explant. No exact measurement was made but enough material was added to insure that some crystals would remain undissolved for the entire period of the experiment. Data on a few cultures in which this was not achieved have been included in the results but with special comment. In dealing with freshly explanted heart, Jacoby, Medawar, and Willmer did not employ embryonic extract in the first passage but appear to have used 0.1 ml. of an equal mixture of cockrel plasma and a solution of the drug in Ringer's. After observation for 72 hours their tissues were washed in Ringer's and subcultured in a plasma-embryonic extract mixture to test

for irreversible changes. Our results do not extend beyond an initial 72-hour period of study.

(2) *Heart explants prepared as in (1) but grown for 24 hours before being treated with crystals.* In similar tests of cultures whose growth capacity had been proven following incubation, the Cambridge group used fibroblasts derived from parietal bone which had been cultured for upwards of four passages. Such material was then grown in Carrel flasks and treated with solutions ranging in concentration to the saturation point. Our results are entirely for crystal-treated hanging-drop preparations of heart explants which had shown good growth for 24 hours.

(3) *Spleen fragments* from one or two day old chicks were prepared according to suggestions derived from Chambers' and Grand's study on the chemotactic reaction of leucocytes to foreign substances in tissue culture.

(4) In a few experiments borings about 1 mm. in diameter were taken from the *rib of adult roosters.* These were made with a drill ordinarily used in performing hypophysectomies in the young rat. When sufficient cancellous tissue is included such explants yield migrating leucocytic elements.

Faulkner and Duncan have reported that sulfonamides could not be detected in appreciable amounts in human blood clots which had been suspended for as much as 240 hours at 37.5° in suitable solutions. While no quantitative determinations were made of the amount of sulfonamide which had penetrated the plasma clot used in tissue cultures, gross microscopic observation proved that crystals of the more soluble members of this group such as sulfanilamide, sulfathiazole and sodium sulfadiazine quickly disappeared after application.

Results

A summary of the results obtained is given in Table I. An average of one untreated control for six experimental cultures was set up for each tissue used. Projection of total growth areas of cultures and other quantitative methods were not employed. The object of the study was rather to test growth *versus* non-growth in the presence of undissolved sulfonamide crystals. A crude yet satisfactory estimation was made, how-

TABLE I.

The Effect of Sulfonamide Crystals Applied Directly To Fibroblasts and Leucocytes Grown in Tissue Culture

Plus signs indicate positive growth; minus signs denote complete inhibition. Symbols used: L—Eli Lilly and Co.; Ld—Lederle Laboratories; M—Merck and Co.; S—Sharp and Dohme. Double symbols indicate that half the cultures were treated with compounds from each firm.

Compound Used	Tissue	Experiment	Individual Results
Sulfanilamide	Heart fresh explant	$II^{L,M}$	— + — — — —
	Heart 24° growth	I^{L}	— ++ — — —
	Spleen	$III^{L,M}$	+ + / — —
	Spleen	IV^{M}	+ / —
	Spleen	VII^{M}	— —
	Rooster rib	$III^{L,M}$	+ + / — —
Sulfapyridine	Heart fresh explant	II^{M}	+ + ++ + ++ +
	Heart fresh explant	VII^{M}	++ ++ / ++ ++
	Heart 24° growth	I^{M}	++ ++ + + ++ / ++ ++ ++ ++ ++
	Spleen	$III^{L,M}$	+ / ++ ++
	Spleen	IV^{M}	+ / ++
	Spleen	VII^{M}	+ / ++ ++
	Rooster rib	V^{M}	+ ++ / — ++ ++
	Rooster rib	VI^{M}	++ ++ / ++ + ++ ++ ++
Sulfathiazole	Heart fresh explant	$II^{L,M}$	— — + — —
	Heart 24° growth	I^{M}	+ / — ++ ++ — —
	Spleen	$III^{L,M}$	++ ++
	Spleen	IV^{M}	++
	Spleen	VII^{M}	— —

Sulfabenamide[S]	Heart fresh explant	II	++ ++ — —
	Heart fresh explant	VII	++ + ++
	Heart fresh explant	VII	++ ++ ++ + ++ ++ ++ ++
	Spleen	VII	++ ++ ++ ++ ++ ++ ++ ++
Sulfaguanidine[Ld]	Heart fresh explant	V	++ ++
	Heart fresh explant	VII	++ + ++ ++
	Heart 24° growth	VII	++ ++ ++ ++ ++ ++ ++ ++
	Spleen	V	++ ++ ++ ++
	Spleen	VII	++ ++ ++
Sulfadiazine[Ld]	Heart fresh explant	V	++ ++ ++ ++
	Heart 24° growth	VII	+ ++ ++ ++ ++ ++ ++ ++
	Heart 24° growth	IX	++ ++ ++
	Spleen	VIII	++ +
	Spleen	IX	+ + ++ ++
Sodium Sulfadiazine[Ld]	Heart fresh explant	V	— —
	Heart fresh explant	VII	— —
	Heart fresh explant	X	— —
	Heart 24° growth	IX	— — — —
	Spleen	V	— —
	Spleen	VII	+ + — —
	Spleen	VIII	— —
	Spleen	IX	— —

ever, of the approximate degree of leucocytic and fibroblastic migration. These are reported by the familiar designation of + and — signs to indicate respectively positive growth and inhibition. The symbol + + + +, for example, was used to indicate the growth of fibroblasts comparable to those of controls after 72 hours of incubation or migration of leucocytes when compared with a control culture incubated for 24 hours.

Sulfanilamide

Typical results obtained by treating freshly explanted embryonic heart are seen in figures 1 and 2. Even when very lightly dusted with sulfanilamide crystals only a very few cells succeeded in growing; only one out of six cultures showing positive results (figure 3).

Cultures having a vigorous outgrowth after 24 hours of incubation were selected and treated with sulfanilamide but due to the high solubility of this drug most crystals disappeared shortly after its application. Figures 5, 6, and 20 illustrate typical results. Continued growth was only observed in one instance, but since no crystals were seen 6 hours after treatment it is believed that the experiment falls outside of the plan of this study. It can be stated confidently that when a quantity of sulfanilamide sufficient to exceed saturation at 38° C. is used, fibroblastic migration will be completely inhibited. Occasionally sulfanilamide crystals were seen to have clumped on the surface of the culture medium (figure 4).

When an amount of crystals sufficient to persist throughout the period of the experiment is used leucocytic migration from chick spleen and rooster rib borings is almost completely inhibited (*c.f.*, figures 7, 8, 9, and 10).

Sulfapyridine

Growth of fibroblasts was active in cultures treated with sulfapyridine, although some retardation was seen in 5 out of 8 cultures treated with crystals of this compound. Figures 11 and 12 show very successful cell migration from heart tissue treated immediately after the explant was prepared.

Figures 13, 14, 15, 16, and 20 illustrate experiments in which sulfapyridine was added to cultures having grown for 24 hours. Extensive migrations of fibroblasts through fields heavily dusted with sulfapyridine crystals were regularly observed.

While migration of leucocytes was not quite as active as in control cultures, sulfapyridine treated spleen fragments (figures 17 and 18) and rooster rib borings (figure 19) always were found to yield positive results.

Sulfathiazole

Growth of fibroblasts from freshly cut embryonic heart was completely inhibited in 4 out of 5 cultures in the presence of sulfathiazole (figures 21 and 22).

Cultures having been grown for 24 hours seemed slightly less damaged although no growth was seen in such preparations whenever sulfathiazole crystals persisted throughout the 72-hour incubation period (figures 20, 23, and 24).

Migration of leucocytes from treated spleen was completely inhibited in 2 out of 5 experiments. Figure 25 shows a relatively broad distribution of these elements in the presence of sulfathiazole 9 hours after the preparation was made.

Sulfabenamide

While pronounced outgrowth of fibroblasts was observed only in one culture (figure 26), 4 out of 6 cultures grew successfully following the application of sulfabenamide to freshly cut chick heart fragments. Significant of this series was the positive chemotatic reaction of the fibroblasts to the crystals as seen in figures 27, 28, and 29.

Following the addition of sulfabenamide crystals to heart cultures in which growth had been in progress for 24 hours no inhibition of cellular activity was seen in any of the preparations so tested (figures 30 and 31).

Figures 32 and 33 show that sulfabenamide does not seem to retard the migration of leucocytes from spleen fragments. Results on all treated spleen cultures rivaled those of the controls.

Sulfaguanidine

Sulfaguanidine shows little tendency to prevent proliferation of fibroblasts when applied directly to freshly cut heart fragments (figure 34), and seems entirely uninjurious when added to cultures which have been grown for 24 hours (figures 35, 36, 37, and 38). Similarly, migration of leucocytes from spleen is unhindered by sulfaguanidine (figures 39 and 40).

Sulfadiazine

Excellent growth of fibroblasts and leucocytes was obtained from heart and spleen, respectively, in the presence of large quantities of sulfadiazine crystals. Growth of fibroblasts from freshly cut heart are shown in figure 41, while the characteristic effect on a 24 hours growth is illustrated in figures 42 and 43. The active migration of leucocytes in a field of sulfadiazine can be seen in a photograph taken at high magnification (figure 44).

Sodium Sulfadiazine

In contrast to sulfadiazine, the sodium salt is characterized by a degree of solubility which caused tissue culture media to swell markedly. This compound showed a tremendous capacity to inhibit both cell types used in this study. Growth of fibroblasts was never observed in any of 6 preparations in which crystals were applied to the freshly cut heart (figure 45) nor in 4 experiments using cultures whose growth had been established following incubation for 24 hours (figures 46 and 47).

Migration of leucocytes typical of untreated spleen cultures occurred to a very slight degree and in only two out of 8 tests.

After approximately 48 hours, cultures saturated with sodium sulfadiazine exhibited new crystal formations (figure 48), most of which were spherical with an actinar pattern.

Discussion and Conclusions

Jacoby, Medawar and Willmer found that growth of freshly explanted heart was inhibited by sulfanilamide at a concentration of approximately 1:150 while active fibroblasts from bone were arrested at 1:100. Such cultures were found to recover following suitable treatment. Macrophages were not found to recover after exposure to concentrations of 1:60 and 1:100. Results obtained in the present study with direct application of crystals confirmed that sulfanilamide at high concentration is damaging to cells.

The Cambridge workers reported that sulfapyridine failed to affect mitosis or migration at a concentration of 70%. Their observation of growth from freshly cut heart explants and of the movements of macrophages in the presence of saturated solutions of sulfapyridine is corroborated by our own experiments.

Even though the solubility of sulfathiazole is not very much greater than that of sulfapyridine this substance has been found relatively more damaging to the various types of cells employed by both Jacoby, Medawar and Willmer (11) and by ourselves. There is evidence that toxic effects are more pronounced and persistent than in the case of sulfanilamide.

Tissue culture studies again agree in the relative non-toxicity of sulfadiazine, although in our hands the highly soluble sodium salt of this compound led to complete inhibition of all cultures employed.

Microscopic observations consistently showed free migration of fibroblasts and leucocytes among crystals of sulfaguanidine. This was also true of sulfabenamide, a derivative described as having a relatively low antibacterial activity but, on the other hand, an extremely low toxicity.

Thus, it will be seen that the compounds used in this study fall into two groups. Sulfaguanidine, sulfadiazine, sulfabenamide and sulfapyridine are characterized by low solubility and an extraordinarily non-damaging effect on cells at concentrations above saturation at 38° C. Sulfathiazole, sulganilamide and sodium sulfadiazine are very much more soluble

and their inhibitory effects increase in degree in the order listed. The Cambridge group have already signaled the fact that the damaging effect of the sulfonamides is proportional to their respective solubility. They argue that this is not due to osmotic phenomena since heart fibroblasts under similar conditions of cultivation show no damage in the presence of 5% solutions of maltose for long periods.

Tissue culture studies give no evidence that sulfonamides stimulate mitosis or cell migration. Results with different types of cells including the epithelial type give consistent results in relation to the various compounds used. Out of these investigations comes the surprise to experimentalists that sulfonamides can be relatively so harmless to cells. In view of this, Jacoby, Medawar, and Willmer have insisted that the bactericidal activity of the sulfonamides should outweigh other considerations when choice is being made for direct application. While some investigators, as Bick, have reported the retardation of healing when sulfonamides are applied to clean wounds, evidence from tissue culture studies support those, such as Crile and Key, who believe that such applications, when not excessive, are desirable.

Summary

Hanging drop cultures of chick: (1) freshly explanted ventricle, (2) ventricle cultivated for 24 hours, (3) spleen fragments, and (4) rooster rib borings, were dusted with crystals of sulfonamides. No evidence of growth promotion was found. Sulfaguanidine, sulfadiazine, sulfabenamide, and sulfapyridine, which are characterized by low solubility, were found to be extraordinarily non-damaging to cells *in vitro*. Sulfathiazole, sulfanilamide, and sodium sulfadiazine were found to be inhibitory in the order listed, which is also the order of their increasing solubility.

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PLATE I

Explanation of Figures

Fig. 1. *Sulfanilamide.* Freshly explanted chick ventricle dusted with relatively few crystals. x60.

Fig. 2. *Sulfanilamide.* The same culture as in figure 1 but after 48 hours of incubation. No migration of fibroblasts has occurred even though all crystals have become dissolved. x60.

Fig. 3. *Sulfanilamide.* A small number of cells are seen to have migrated from a fragment of ventricle 72 hours after treatment with crystals. However, no undissolved crystals remain in the culture. x60.

Fig. 4. *Sulfanilamide.* After all of the original crystals applied to this culture had become dissolved new crystal formations appeared. 72 hour culture. x180.

Fig. 5. *Sulfanilamide.* Chick ventricle culture incubated for 24 hours. Photographed immediately after treatment. x60.

Fig. 6. *Sulfanilamide.* Same culture as in fig. 5, but 48 hours later. Migration of fibroblasts has ceased and the cells have become rounded. x60.

Fig. 7. *Sulfanilamide.* Fragment of spleen 9 hours after treatment showing restricted migration of leucocytes. x40.

Fig. 8. *Control* for cultures seen in figs. 6, 17 and 25. Untreated culture after 9 hours of incubation. x40.

Fig. 9. *Sulfanilamide.* Edge of spleen culture showing migration of relatively few leucocytes 5 hours after treatment. x60.

Fig. 10. *Control* for cultures shown in figure 9 and 18. Untreated spleen culture after 5 hours of incubation. x60.

Fig. 11. *Sulfapyridine.* Growth of fibroblasts after 72 hours of incubation. Crystals were applied at the time of explanation. x60.

Fig. 12. *Sulfapyridine.* Another culture prepared like that shown in fig. 11. Fibroblasts have migrated through a field heavily dusted with crystals. x60.

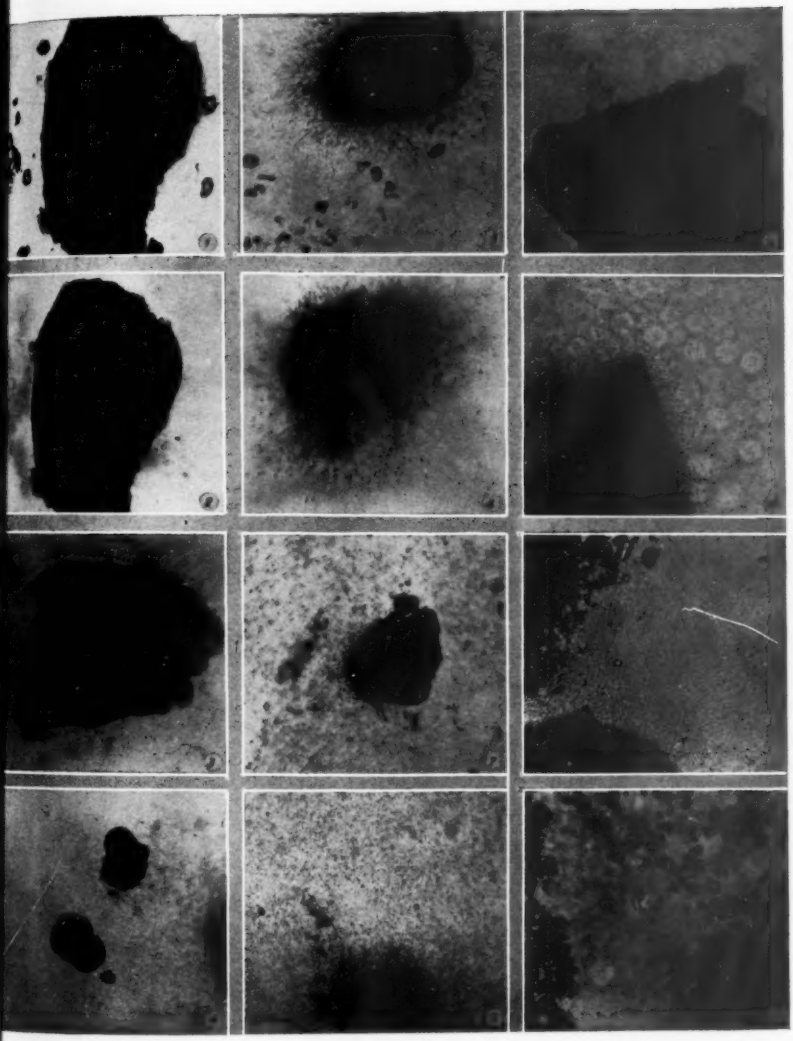

PLATE I

PLATE II

Explanation of Figures

Fig. 13. *Heart culture* after 24 hours of incubation, showing vigorous growth of fibroblasts. x60.

Fig. 14. *Sulfapyridine.* Same culture as in fig. 13 but photographed 45 minutes after having been dusted with a large quantity of crystals. x60.

Fig. 15. *Sulfapyridine.* Same culture as in fig. 13 but 46½ hrs. later. The photograph was made in the plane of the crystals. x60.

Fig. 16. *Sulfapyridine.* Photograph of the same area as in fig. 15 but focused in the plane of migrating fibroblasts whose activities do not appear to have been hindered by the presence of the crystals. (The control for this experiment is shown in fig. 20.) x60.

Fig. 17. *Sulfapyridine.* Crystals applied at the time of explanation can be seen in lower edge of a spleen fragment. Leucocytic migration appears to have been active. x40.

Fig. 18. *Sulfapyridine.* Crystals in relation to leucocytic activity from a spleen explant 5 hours after treatment. Compare with control culture in fig. 10. x60.

Fig. 19. *Sulfapyridine.* Migration of leucocytes from a rooster rib boring (lower right) in the presence of crystals (left side) after 24 hours of incubation. x60.

Fig. 20. *Control* for explants shown in figs. 6, 16 and 24. Untreated culture after 72 hours of incubation. x60.

Fig. 21. *Sulfathiazole.* Initial photograph of freshly prepared heart explant treated with crystals. x60.

Fig. 22. *Sulfathiazole.* Same culture as in fig. 21 but 72 hours later, showing typical results obtained in such experiments. Crystals have almost all dissolved and no outgrowth of fibroblasts has occurred. x60.

Fig. 23. *Sulfathiazole.* 24 hour heart culture 35 minutes after having been dusted lightly with crystals. x60.

Fig. 24. *Sulfathiazole.* Same culture as in fig. 23 but 47 hours later, showing complete inhibition of movement and rounding of cells. x60.

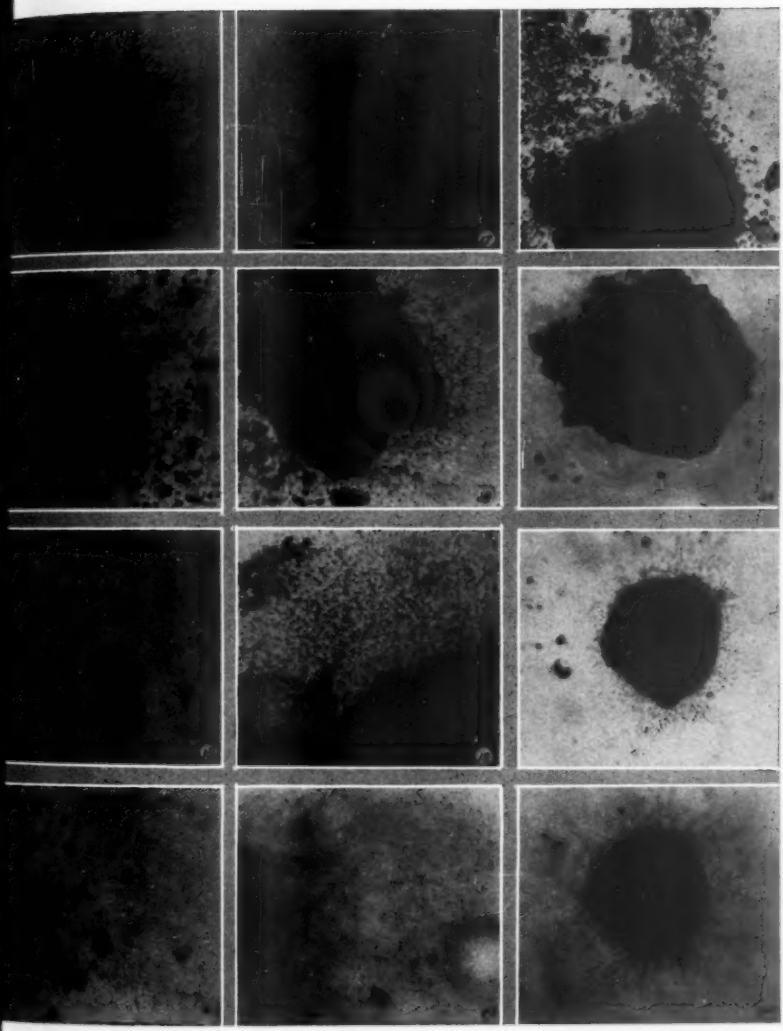

PLATE II

PLATE III

Explanation of Figures

Fig. 25. *Sulfathiazole.* Spleen fragment with crystals at one pole. Some leucocytic migration has occurred. Compare with cultures in figs. 8 and 17. x40.

Fig. 26. *Sulfabenamide.* Crystals applied directly to freshly explanted chick ventricle have not retarded migration of fibroblasts following 72 hours of incubation. x60.

Figs. 27, 28 and 29. *Sulfabenamide.* Reactions of fibroblasts from chick ventricle to crystals. Note evidences of chemotactic reactions. 72 hour culture. x180.

Fig. 30. *Sulfabenamide.* 24 hour heart culture photographed immediately after treatment with a large quantity of crystals. x60.

Fig. 31. *Sulfabenamide.* Same culture as in fig. 30 but 48 hours later, showing that fibroblasts have continued their growth through a field of crystals. x60.

Fig. 32. *Sulfabenamide.* Active migration of leucocytes through a field of crystals; from a spleen fragment. Culture incubated for 17 hours. x60.

Fig. 33. *Sulfabenamide.* Same culture as in figure 32 but at higher magnification to show the relation of leucocytes to the crystals. x180.

Fig. 34. *Sulfaguanidine.* Migration of fibroblasts through a field of crystals 72 hours after application to freshly cut heart. x60.

Fig. 35. *Sulfaguanidine.* Growth of fibroblasts in a heart culture after 24 hours incubation. Photograph taken immediately after application of crystals. x60.

Fig. 36. *Sulfaguanidine.* Same culture as in fig. 34, but photographed 48 hours later. x60.

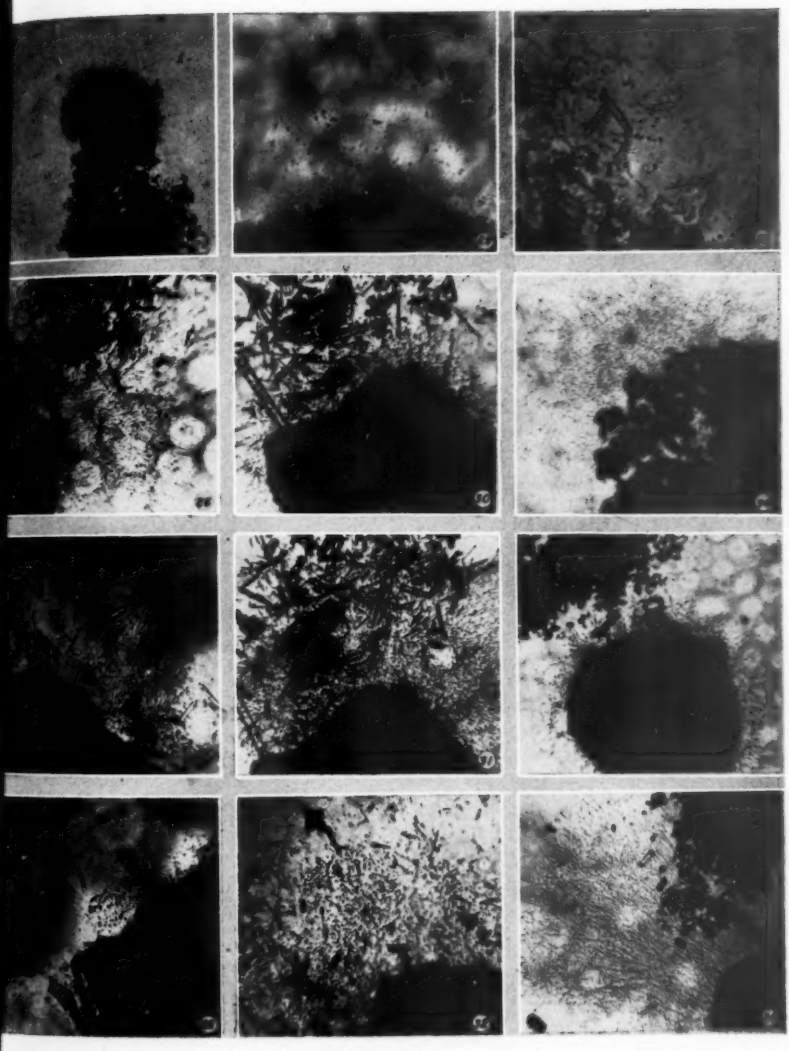

PLATE III

PLATE IV

Explanation of Figures

Fig. 37. *Sulfaguanidine.* Growth of fibroblasts in a heart culture after 24 hours incubation. Photograph taken immediately after application of crystals. x60.

Fig. 38. *Sulfaguanidine.* Same culture as in figure 37 but photographed 48 hours later to show active migration of fibroblasts through a field heavily dusted with crystals. x60.

Fig. 39. *Sulfaguanidine.* Leucocytes from spleen migration in the presence of crystals after 10 hours of incubation. x60.

Fig. 40. *Sulfaguanidine.* Same culture as in fig. 39 but at higher magnification, showing proximity of leucocytes to crystals. x180.

Fig. 41. *Sulfadiazine* crystals on freshly cut chick heart showing outgrowth of fibroblasts after 72 hours of incubation. x60.

Fig. 42. *Sulfadiazine* growth of fibroblasts in a heart culture after 24 hours incubation. Photograph taken immediately after application of crystals. x60.

Fig. 43. *Sulfadiazine.* Same culture as in fig. 42 but photographed 48 hours later showing relation of fibroblasts to the field of crystals. x60.

Fig. 44. *Sulfadiazine.* Same culture as fig. 43 but at higher magnification. x180.

Fig. 45. *Sodium Sulfadiazine.* Freshly cut heart following treatment at the time of explanation. Typical, complete inhibition with reformation of crystals after 72 hours of incubation. x60.

Fig. 46. *Sodium Sulfadiazine.* Growth of fibroblasts in a heart culture after 24 hours of incubation. In spite of having added a quantity of crystals sufficient to cause the clot to swell markedly, little evidence of the compound remained when the culture was photographed. x60.

Fig. 47. *Sodium Sulfadiazine.* Same culture as in fig. 46, 48 hours later. No further growth of fibroblasts has occurred and new crystals have precipitated in the medium. x60.

Fig. 48. *Sodium Sulfadiazine.* Crystals which have reformed in the culture shown in fig. 47 photographed at higher magnification. x180.

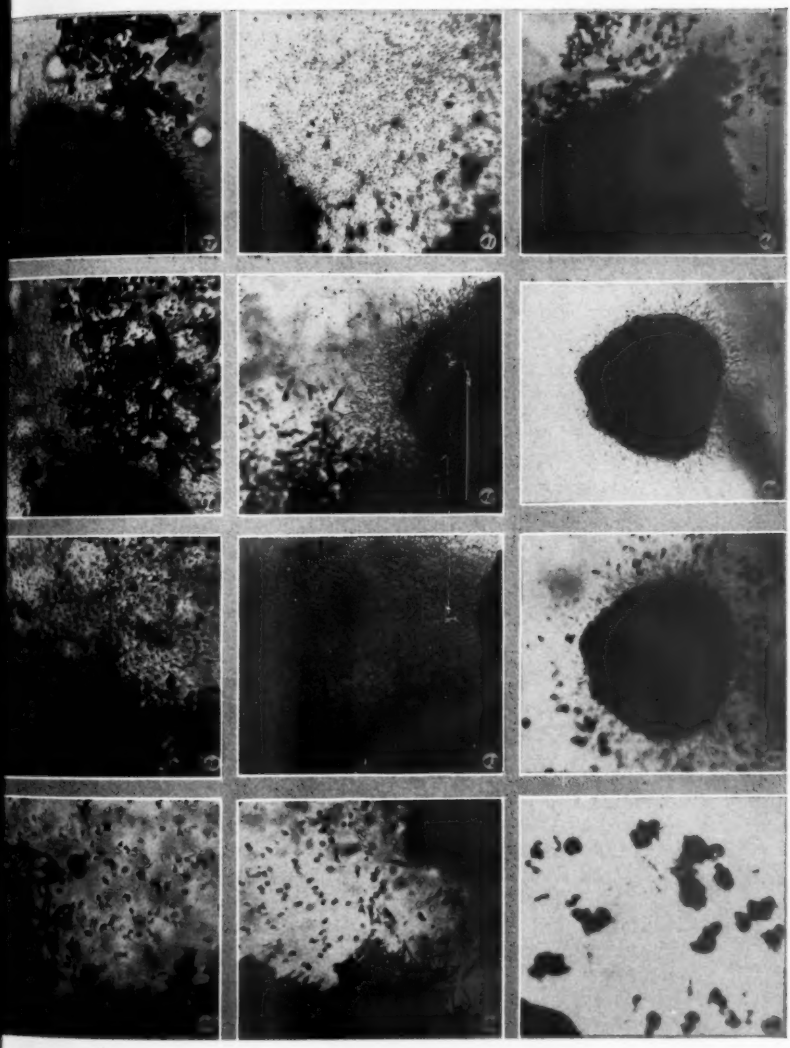

PLATE IV

CALLING ATTENTION TO:

Items of Possible Interest to Friends of Our Effort

1. Hold Everything: Right after H. Raistrick & Co.'s strong boost for patulin for the common cold (*Lancet*, 245:625, Nov. 20, 1943) come C. H. Stuart-Harris & Co. saying 'tain't so (*Ibid.*, p. 684, Nov. 27, 1943). Well, relax and enjoy the wit and wisdom of O. H. P. Pepper's Fever Curve of Therapeutic Fads (*Amer. J. Med. Sci.* 206:703, Dec., 1943).

2. Among New Books: American Psychiatric Asso. announces *One Hundred Years of American Psychiatry*, with 15 chapters by various historians (Columbia Univ. Press, N. Y., 1944). Also from the same excellent press comes *New Goals for Old Age*, well edited by G. Lawton (N. Y., 1943). 2nd edition of G. L. Jenkins and W. H. Hartung's *Chemistry of Organic Medicinal Products* appears expanded and improved (John Wiley & Sons, N. Y. 16, 1944). T. E. Keys' admirable series of essays on *The Development of Anesthesia* would make a swell booklet, after collection from *Anesthesiology* (Vols. 3 and 4) in which they originally appeared. Allergists might note G. Erdtman's *Introduction to Pollen Analysis* (Chronica Botanica, Waltham, Mass., 1943). W. H. Schopfer's *Plants and Vitamins* has been translated by N. L. Noecker (Chronica Botanica, Waltham, 1943). *Frontiers in Cytochemistry* is a honey symposium in honor of R. R. Bensley, edited by N. L. Hoerr (*Biol. Symposia* X, Cattell Press, Lancaster, Pa., 1943). P. B. Hoeber offers 4 likelies: G. Draper & Co.'s *Human Constitution in Clinical Medicine;* O. Glasser & Co.'s *Physical Foundations of Radiology;* R. W. Nauss's *Medical Parasitology and Zoology;* B. P. Babkin's *Secretory Mechanisms of the Digestive Glands* (N. Y., 1944). J. B. Lippincott issue 23rd edition *The Dispensatory of the United States of America*, 1881 pages, edited by H. C. Wood & Co. for $15 (Philadelphia, 1943). Bremer's *Histology* has been revised by H. L. Weatherford (Blakiston, Phila., 5, 1944). For $35, W. B. Saunders Co. offers H. L. Bockus' 3 Vol. *Gastro-Enterology* (Phila. 5, 1943). They also present R. P. McCombs neat *Internal Medicine* (Phila. 5, 1943). For $2 one may get I. D. Cardiff's *A Million Years of Human Progress* (Dorset, N. Y., 1943). Pithy is M. Greenwood's Linacre Lecture, *Authority in Medicine* (Macmillan, N. Y., 1943). Helpful is W. D. Postell's *Medical Bibliography* (New Orleans, 1943).

3. Shock: J. Davis says watch A. Cournand & Co.'s studies on circulation in clinical shock, defined as end stage of circulatory failure, and noting hemodilution in traumatic and hemorrhagic shock with rapid circulatory failure and possible vasomotor collapse, but hemoconcentration in burns and abdominal injury (*Surgery*, 13:964, 1943). Y. Henderson

struts perky review on tonus and denopressor mechanism and clinical physiology of major mode of death (*Medicine*, 22:223, 1943), and then raises hob with Council on Physical Therapy of A. M. A. over the return of the pulmotor as a "resuscitator" a back-step toward the death of thousands (*Science*, 98:547, Dec. 24, 1943). How about the H-H Inhalator? R. A. Cleghorn finds mortality in dogs from standard severe bleeding is less at 72° F. than at colder or warmer temperatures (*Canad. Med. Assoc. J.* 49:362, 1943). R. L. Noble & Co. induce trauma resistance in rats, by gradually increasing trauma, and find less biochemical change in resistant than in normal animals (*Amer. J. Physiol.* 138:346, 1943; *Proc. Soc. Exp. Biol. Med.* 54:249, 1943). W. H. Cole & Co. find no correlation between plasma protein and blood specific gravity in gravity shock in rabbits (*Ibid.*, p. 215).

4. Tooting Our Own: R. Gregory, P. Ewing et al., in addition to stirring up much on hypertension (*Texas Rep. Biol. Med.* 1:167, 1943 and more to come) show insulin glucose and pyruvate have no effect on rate of alcohol metabolism (*Proc. Soc. Biol. Med.* 54:206, 209, 1943). A. H. Hanszen and W. Selle find adsorbents in diet inhibit butter yellow liver cancer (*Ibid.*, p. 225). G. Emerson notes neostigmine plus dilantin protect rats against severe anoxia (*Ibid.*, p. 252). L. L. Lumsden, C. A. Nau and F. M. Stead are honest about our food poisoning (*Pub. Health Rep.* 58:1497, 1943). E. J. Poth describes succinyl and phthalyl sulfathiazols as intestinal antiseptics (*Texas S. J. Med.* 39:369, 1943).

5. Etc.: J. W. Knutson and W. D. Armstrong show some beneficial effect from 10 topical applications 2 per cent NaFl sol. in preventing dental caries (*Pub. Health Rep.* 58:1701, 1943). L. J. Davis offers helpful survey of hemolytic anemias (*Edin. Med. J.* 50:589, 1943). W. T. Longcope discusses serum sickness from sulfonamides (*Med.* 22:351, 1943).

6. Journals: Washington (DC) Institute of Medicine issues (at $9 a year) new bimonthly *General Practice Clinics*, which is merely an abstract and review service. *Bulletin US Army Medical Department* now appears monthly. Recent issue (#72, p. 50, 1944) carries report by S. G. Page on sulfaguanidine in bacillary dysentery, indicating relatively high absorption of the drug but with little toxic reaction. *Texas Reports on Biology and Medicine* may come along more on time and with improved format from University Press.

7. From Our Fighting Allies: S. Sze offers divertisement on how Drs. H. Macartney, P. Mason, and J. Cantlie rescued Dr. Sun Yat Sen in London in 1896 when he was about to be deported to China for execution (*Chinese Med. J.*, 61:172, 1943). V. D. Kostenko finds ascorbic acid content of plants much increased by growth at high altitudes, thus suggesting function as hydrogen carrier to increase cellular respiration, and indicating value of high administration to fliers (*Compt. rend. Acad. Sci. USSR*, 38: 42, 1943) D. K. Henderson and Co. survey (favorably) electrical convulsion therapy (*Edin. Med. J.*, 50:641, 1943). A. J. M. Sinclair gives solid discussion of psychiatric casualties from jungle fighting (*Med.*

J. Australia, 2:453, Dec. 4, 1943). C. D. da Silva and J. P. G.d'Alambert review syndrome of internal frontal hyperostosis (*Arq. Assist. Psico. Est. Soa Paulo*, 8:55, 1943).

8. From Our Friends: B. A. Houssay says thyrodiectomy has no effect on development of diabetes, but that thyroid administration may cause severe reactions in experimental diabetes (*Rev. Soc. Argentina Biol.*, 19:94, 1943). E. F. Lascano shows both arteries and anastomoses supplying Tawara's node and bundle of his and its branches run in same direction as wave of excitability (*Rev. Argentina Cardiol.*, 10:23, 1943).

9. From the Amazingly Neutral Swiss: Special number of League of Nations *Chronicale of the Health Organization*. 1st since May, 1940, reports continuance of *Weekly Epidemiology Record and Bulletin of the Health Organization*, and progress on international pharmacopeia. C. Montigel and F. Verzar report interesting series of studies on carbohydrate metabolism after adrenalectomy showing that desoxy-corticosterone promotes much glycogen storage in liver and muscles (*Helv. Physiol. Pharmacol. Acta*, 1:137, 1943). N. Scheinfinkel suggests that thiamin in combination with a cardiac enzyme inactivates acetylcholine (*Ibid.*, p. 149).

10. From Our Enemies: H. Hofmann offers intriguing study on permeability changes from narcotics and analeptics (*Arch. Exper. Path. Pharmakol.*, 201:529, 1943). I. Traina of Genoa offers a photometric method for estimating atabrine in urine and feces (*Biochem. Zeitschr.*, 315: 111, 1943).

11. Antibiotics: I. R. Hooper and Co. report (*Science*, 99:16, Jan. 7, '44), that S. Waksman's "clavacin" from Aspergillus clavatus (*J. Bact.*, 45: 233, 1943) is identical with H. Raistrick's "patulin" from Penicillium patulum (*Lancet*, 245:633, 1943). D. L. Augustine finds penicillin sodium effective in relapsing fever but not in trypanosomiasis (*Science*, 99:19, Jan. 7, '44).

12. And Further: D. Nachmansohn and Co. continue fine work on action potentials and enzyme activity, and find new enzyme, choline acetylase, which forms acetylcholine in presence of adenosine triphosphate (*J. Neurophysiol.*, 6:383, 397, 1943). N. Shock offers meticulus studies on homeostatic adjustments after exercise (*J. Gen. Physiol.*, 27:143, 1944). Yale Lab of Applied Physiology becomes School of Alcohol Studies and goes behavioristic in S. D. Bacon's sociology and the problems of alcohol (*Quart. J. Stud. Alc.*, 4:387, 1943). E. M. Loeb reviews primitive intoxicants (*Ibid.*, p. 402). Hot: J. P. Scott demonstrates experimentally that differences in social organization are caused by differences in social behavior, by conditioning same male mice alternately to fighting or to peaceful behavior. (*Science*, 99:42, Jan. 14, '44). L. du Nouy properly closes (*Ibid.*, p. 38) the discussion on apparent time acceleration with age, by reference to his *Biological Time* (Macmillan, New York, 1937). A. W. Winkler and Co. discuss sea water toxicity (*J. Clin. Invest.* 23: 103, 1944). J. B. de C. M. Saunders and Co. continue detailed study of amelogenesis (*J. Amer. Col. Dent.* 10:241, 1943).